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Innovative Technologies for Remediation of Soils and Groundwater for the Utah Test and Training Range

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MBI International and Utah State University together developed a two pronged strategy to reduce or eliminate TCE in Soils. USU's effort concentrated on the assessment of the effectiveness of microbial augmentation in stimulating the complete dechlorination of TCE in a simulated aquifer system constructed of soil and groundwater from Hill AFB. MBI International's effort focused on the evaluation of surfactant systems in stripping TCE from contaminated soil. This final report documents the results of both efforts..

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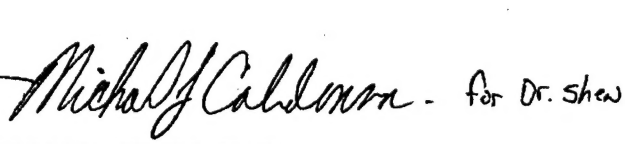

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ABSTRACT

Hill Air Force Base (AFB), Utah, has documented contamination of soil and water with chlorinated solvents such as trichloroethylene (TCE).

MBI International in collaboration with Utah State University developed a two-pronged strategy to reduce or eliminate TCE in soils. Utah State University's effort concentrated on the assessment of the effectiveness of microbial augmentation in stimulating the complete dechlorination of TCE in a simulated aquifer system constructed of soil and groundwater from Hill AFB. Bioaugmentation using spiked microorganisms showed significant TCE degradation. TCE degradation could not be stimulated by the addition of a carbon donor alone when compared to controls. Water quality changes in carbon donor amended reactors without bioaugmentation led to reducing conditions that were evident from releases of dissolved iron and arsenic, the reduction of sulfate and the generation of large quantities of methane. Quantitative data generated in the complete culture augmentation study confirmed the need for a combined carbon donor and microbial amendment for the stimulation of TCE degradation. The Bachman Road culture plus emulsified oil appeared to provide the best treatment.

MBI International's effort focused on the evaluation of surfactant systems in stripping TCE from contaminated soil. Surfactant performance was evaluated by testing the equilibrium distribution of TCE in surfactant solutions, performing shake-vial separation equilibrium, and simulating surfactant flooding in column simulations. The results show micro-emulsion blends holding up to 40 mg TCE ml^{-1} in the water phase and blends of surfactant with salt and isopropanol were made temperature insensitive. Recovery of TCE from single extraction shake-vial tests ranged only 2-17%. However, precipitation of various surfactants with the site waters led to some plugging problems resulting in only a few tenths of one percent TCE recoveries from flow-through column.

Contents

List of Figures

List of Tables

Summary

The Air Force requires advanced technologies that facilitate the conduct of combat operations and operations other than war by Air Expeditionary Forces, and provides opportunities for reduction of the total ownership costs of combat systems. Hill Air Force Base (AFB) in Utah has documented environmental challenges resulting from past operations. These include contamination of soil and water with chlorinated solvents such as trichloroethylene (TCE), 1,1,1-trichloroethane (TCA), and perchloroethylene (PCE). Additional polychlorinated biphenyl (PCB) contamination has been identified in a reconditioning site for diesel locomotives. Hill Air Force Base is regulated under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the Resource Conservation and Recovery Act (RCRA) provisions of Federal pollution legislation and the site is operating under a 100-year compliance schedule to mitigate the effects of TCE dispersion to the surrounding groundwater. The overall project was to promote two innovative remediation technologies, groundwater amendment with a biological culture and separation of pollutants by surfactant washing and test their applicability to the Hill AFB site. Investigation of the benefits of soil and groundwater amendment with two biological cultures compared to a matrix of physical-chemical amendments for the treatment of TCE was undertaken by Utah State University (USU). A novel surfactant system capable of stripping oily pollutants such as diesel fuel and PCBs from soil was tested with Hill AFB samples by MBI International (MBI). The work under the project was comprised of three tasks:

Task 1: Preparation and Optimization of TCE Degrading Cultures
Task 2: Microcosm Testing of Augmented Soils
Task 3: Separation of Pollutants from Soils by Surfactant Washing

The work under Task 1, Preparation and Optimization of TCE Degrading Cultures, was subcontracted to the RETEC Group, Inc. (RETEC) (Lansing, MI). Two cultures, a granular culture developed by MBI and the "Bachman Road" culture, both known to degrade TCE, were supplied by RETEC for use by USU in microcosm and soil column tests. The objectives of RETEC's work were growth of a sufficient mass of MBI's granular TCE-dechlorinating culture for use in the planned testing and determination of the costs of growing the culture at the scale needed for bioaugmentation at the field site. Testing for maximum rates of transformation achievable with the granules was also performed to allow a basis of comparison of rates achieved during the microcosm testing. RETEC also supplied a dispersed cell culture capable of dechlorinating PCE and other chlorinated ethenes, the Bachman Road culture for comparative testing.

Results from the RETEC studies and microcosm testing conducted at Utah State University show that the MBI granular culture is capable of completely dechlorinating TEC and its daughter partial dechlorination products (see Appendix B).

A feasibility study was conducted on the cost of growing sufficient inoculum for field site testing (see Appendix B). The use of skid-mounted USAB reactors at the site would eliminate the logistics and costs of transporting large volumes of the inoculum over long distances or the cost of a permanent on-site system. The cost of the equipment skids, not inclusive of costs for temporary housing for the structure, shipment and installation costs, and costs for start-up decommissioning and demobilization, were estimated at $580,000. The estimated cost of operation is $140,000, inclusive of manpower, analytical and chemicals, but exclusive of costs for design, installation and operation of the pumps, wells and other ancillary equipment needed. Other significant and necessary costs were beyond the scope of the study. A considerably more detailed costs analysis is required to better define the true economic feasibility of this approach. This preliminary study does indicate that bioaugmentation is a potential option for sites such as Hill AFB where it appears that biostimulation is not effective.

Work under Task 2, Microcosm Testing of Augmented Soils, was undertaken by Utah State University (Appendix C). The focus of the work performed by USU was to assess the effectiveness of the augmentation of microbial inoculum in stimulating the complete dechlorination of TCE in simulated aquifer systems constructed of soil and groundwater from Hill AFB. The purpose of the study was to provide proof-of-concept and preliminary design data for the use of a microbial amendment to optimize chlorinated hydrocarbon-impacted groundwater remediation at Operating Unit (OU) 5 at Hill AFB. The study was conducted as a complement to an ongoing Hill AFB-funded project to assess carbon donor addition alone as a means of stimulating dechlorination in indigenous microbial populations. Results of the carbon donor study have been incorporated into USU's final report (Appendix B) so that the response of the aquifer system to microbial amendment could be fully demonstrated relative to carbon donor stimulated systems used as active controls.

Specific objectives of the study were (1) to verify that two microbial consortium, the MBI granular culture and the "Bachman Road" culture, are capable of providing completed anaerobic dehalogenation of TCE in microcosms constructed from soil and groundwater collected from OU5 at Hill AFB, when these microcosms are amended with suitable carbon substrate; (2) to evaluate the comparative effectiveness of various soluble and slow release carbon substrates to stimulate TCE dechlorination by these microbial consortium; and (3) to evaluate the effectiveness of zero valent iron to provide

TCE dechlorination in this site groundwater for comparison with accelerated biological means for chlorinated hydrocarbon dechlorination.

The study objectives were met by completion of laboratory batch microcosm studies using soil and groundwater collected from OU5 at Hill AFB. There were four sets of experiments that were designed to evaluate the effectiveness of microbial amendment in stimulating TCE dechlorination and to investigate ZVI-TCE reactions in the OU5 aquifer material. The experiments included a preliminary microbial inocula dilution study designed to determine the optimal mixed culture dilution required to produce dechlorination in spiked microcosms; a preliminary range-finding study to determine rate of TCE transformation in response to a variety of carbon donor amendments; a complete culture augmentation study to determine optimal carbon donor and microbial culture amendment that could be used to accelerate TCE dechlorination at OU5; and a detailed analysis of TCE interaction with Peerless ZVI and the effects of iron corrosion on resultant TCE degradation rates.

Results of the various phases of the study show chlorinated hydrocarbon degradation in the dilution study and the range-finding experiment sufficient to be relevant to remediation system scale-up. ZVI-TCE degradation was found to be significantly greater than TCE concentration reductions in the soil control reactors.

The complete culture augmentation study indicates that without bioaugmentation, significant TCE degradation cannot be stimulated with the addition of carbon donor. TCE degradation rates were not significantly different than the control treatment for oil amendments. Whey stimulated dechlorination only minimally and resulted in a time period that was too long to be acceptable for application in site remediation.

Water quality changes in carbon donor amended reactors without bioaugmentation lead to reducing conditions that were evident from the releases of dissolved iron and dissolved arsenic, the reduction of sulfate and the generation of large quantities of methane in various settings. These reduced conditions did not result in TCE degradation, supporting the conclusion that TCE degradation at OU5 will not be possible without bioaugmentation used along with carbon donor addition.

Quantitative data generated in the complete culture augmentation study confirm the need for and benefit of the use of combined carbon donor and microbial amendment for the stimulation of TCE degradation at the Hill AFB OU5 site. Based on the data generated the "Bachman Road" culture plus emulsified oil appeared to provide optimal treatment.

TCE reaction with ZVI in the groundwater indicated that the reaction slows significantly over time, leading to the development of a dual reaction expression to describe TCE-ZVI interaction. The relationship has a fast degradation reaction that is followed by a much slower degradation reaction. The reactions that occur have the potential to limit the effectiveness of a ZVI wall at OU5 and further analysis of the long-term TCE degradation rates that can be provided by ZVI should be carefully evaluated before a full-scale iron wall is implemented at this site.

The work effort under Task 3, Separation of Pollutants from Soils by Surfactant Washing, performed by MBI International, involved surfactant washing or flooding for groundwater and soil remediation. Surfactant enhanced soil flushing is one alternative means of improving the efficiency of pump and treat technology (moving water from the contaminated zone to an *ex situ* mechanical or biological treatment system) by improving the solubility or mobility pollutants in aqueous systems. However, soil flushing and soil washing remain plagued to this day by the creation of strong emulsions that do not allow for surfactant recovery. This project uses a low emulsion-forming surfactant system (Rhema Super Matrix) and blends of it that have the unique capacity to reject oils/TCE to a clear and separate layer leading to potential for recycling of the surfactant. Rhema, a blend of several surfactants in an alkaline builder, when mixed with oil forms a relatively small and unstable emulsion that readily breaks up leaving only a small amount of oil in the surfactant solution. In addition, three anionic surfactants, sodium lauryl sulfate (SLS), Dowfax, and aerosol, and two non-ionic surfactants, Witconol and Triton were studied for comparison. As the various surfactants were removed from the study at the various screening levels, a blend of Rhema Super Matrix and SLS was created that has interesting temperature stability properties.

Surfactant performances with respect to equilibrium distribution of TCE in surfactant solutions, shake-vial separation equilibrium, and ability to remove TCE in surfactant flooding in column simulation were evaluated. Equilibrium distribution tests involved thorough mixing of different compositions of surfactant, salt, and isopropanol and the amount TCE in the emulsion determined by gas chromatography. The different surfactant blends were also used to extract TCE from spiked Utah soil samples in shake-vial tests. In addition, the surfactant mixtures were used to simulate surfactant flooding in columns packed with spiked Utah soil samples. In all cases, the amount of TCE extracted was determined by headspace gas chromatography.

The results were interesting, especially with respect to temperature. Micro-emulsion blends that could hold up to 40 mg TCE mL^{-1} in the water phase and blends of surfactant with salt and isopropanol that could be made temperature insensitive were made. In general, Rhema and Rhema blends showed better TCE recoveries at low temperature (10 °C, estimated as

groundwater temperature) than at elevated temperatures whereas the opposite is true for Aerosol and SLS, the other two remaining surfactants in the study. In particular, blends of Rhema and SLS (5:1 and 6:1, Rhema:SLS) showed even greater TCE recoveries at 10 °C. Recovery of TCE from single shake-vial extraction tests using the various surfactant mixtures ranged only 2-17%. However, precipitation of various surfactants with the site waters led to some plugging problems resulting in TCE recoveries from flow-through column tests of only a few tenths of one percent with several pore volume turnovers.

The performance of Rhema Super Matrix in the remediation of TCE contaminated soils was comparable to other surfactants in the study. However, Rhema and Rhema blends tend to form unstable emulsions making recovery and reuse of the surfactant feasible. They also tend to perform better at lower temperatures. However, more work is needed to optimize the surfactant system to make it commercially viable.

1.0 Introduction

MBI International, in collaboration with Utah State University, developed a two-pronged strategy to reduce or eliminate TCE in soils. Previously, MBI had developed a granular microbial culture known to degrade TCE. Under a subcontract from MBI, The RETEC Group, Inc., the granular microbial culture and the "Bachman Road" culture, also known to degrade TCE, were produced and delivered to Utah State University. USU's effort concentrated on the assessment of the effectiveness of microbial augmentation in stimulating the complete dechlorination of TCE in a simulated aquifer system constructed of soil and groundwater from Hill AFB. A second approach employed the use of a novel surfactant system to strip oily pollutants from soil with an objective of rejecting oils to a clear and separate layer, leading the potential recycling of the surfactant.

The report is organized as follows:

1. The main body of the report focuses on the MBI International effort under Task 3, Separation of Pollutants from Soils by Surfactant Washing, to employ its novel surfactant system to the removal of TCE from contaminated soils from Hill AFB.
2. Appendix B summarizes the effort by The RETEC Group, Inc. under Task 1, Preparation and Optimization of TCE Degrading Cultures, in the production of the MBI granular culture and the "Bachman Road" culture for use at Utah State University and their economic analysis on the production methods. This work was performed under a subcontract from MBI International.
3. Appendix C summarizes the effort by Utah State University under Task 2, Microcosm Testing of Augmented Soils, to use microbial augmentation to stimulate the degradation of TCE. This work was also performed under a subcontract from MBI International.

1.1 Subject:

The Air Force requires advanced technologies that facilitate the conduct of combat operations and operations other than war by Air Expeditionary Forces, and provide opportunities for reduction of the total ownership costs of combat systems. More specifically, methods of sensing contaminants, providing active and passive protection, and innovative decontamination technologies for force protection at fixed and forward bases are required. Hill AFB, Utah, has documented environmental challenges resulting from past operations. The primary contamination of soil and water is with chlorinated solvents such as (TCE). Other solvents include 1,1,1-trichloroethane (TCA) and perchloroethylene (PCE). Additional contamination with more complex materials such as polychlorinated biphenyls (PCBs) has been identified in a reconditioning site for diesel locomotives.

1.2 Purpose:

In situ groundwater and soil treatment programs have seen a tremendous increase in popularity in the last few years. In a survey conducted in 1993 (Wilson and Kaback, 1993), 105 horizontal pollution treatment wells were catalogued. By 2000, the number of active horizontal wells had increased to 1,142 (Concurrent Technologies Corporation, 2002). Horizontal wells have been used for groundwater extraction (pump and treat) (34%), soil vapor extraction (28%), air sparging (5%), free product recovery (21%), bioventing/bioremediation (3%), chemical oxidant and heat treatment, and hydraulic containment (9%). In a series of 274 case study abstracts (EPA 1995, 1997, 1998, 2000, and 2001) on remediation projects, EPA reported on a number of classes of treatment. The 1995 report focused on groundwater treatment for product recovery (11 reports), bioremediation (9 reports), soil vapor extraction (10 reports) and thermal treatments (7 reports). The 1998 report focused on groundwater pump and treat for low concentration materials (27 reports) and on-site incineration (15 reports).

Classical pump and treat (moving water from the contaminated zone to an *ex situ* mechanical or biological treatment system) methods have been less successful than desired, especially in the clean up of non-aqueous phase liquid (NAPL) (West and Harwell, 1992, Haley *et. al.*, 1991; EPA 1995a). The pump and treat process has been limited to treating water downstream of the NAPL source due to low solubility of the pollutant in the water phase. Many of the pump and treat processes rely on activated carbon to capture the offending organic pollutant from the pumped water stream (Hayes *et. al.*, 1996). Remediation times may exceed decades (Josef *et. al.*, 1998).

Surfactant enhanced soil flushing is seen as one means of improving the efficiency of pump and treat technology by improving the solubility or mobility of NAPL. As of 1997, there had been 26 documented field trials for NAPL recovery using surfactants (CH_2Mhill, 1997). At that time, the range of target compounds ranged from dense non-aqueous phase liquid (DNAPL) (TCE, PCE, carbon tetrachloride and wood treating chemicals) to light non-aqueous phase liquid (LNAPL) (jet fuel, cutting oil, PCBs, and hydraulic oil) to sorbed fuels and PCB's. A collection of 84 soil-flushing projects was compiled by the Ground-Water Remediation Technologies Analysis Center (GWRTAC) (1998). An estimated 54% of the respondents used surfactant enhancements and an additional 9% used co-solvents in the flushing regimes. Another 18% used other enhancements such as sugars, electrolytes, or solvents. Approximately 25% of the case studies were full-scale treatment, 50% were pilot demonstrations, and 25% were at the bench or research phase.

At many sites, NAPL exists in the vadose zone as both free-product and as saturated soil or ganglia. The challenge is to mobilize both the free product

and make the saturated soil release the organic. It is generally recognized, however, that it is critical to the control of any polluted groundwater to gain control over the source (Wilson and Clarke, 1994). The removal of TCE and related chlorinated hydrocarbon source zones at United States Department of Defense (DoD) facilities is the top priority of the Strategic Environmental Research and Development Program (SERDP)/ Environmental Security Technology Certification Program (ESTCP) program (SERDP/ESTCP, 2002).

Although the use of surfactant enhanced oil recovery had been known from the 1920's (Beckstrom and Van Tuyl, 1927), perhaps the first published report of surfactant flushing was a series of studies to optimize surfactants for the removal of gasoline from soil (Texas Research Institute, 1979, 1985). These authors used a blend of anionic and non-ionic surfactants. A series of later studies (Ellis *et. al.*, 1984 and Ellis *et. al.*, 1985) focused on the flushing of PCB's, chlorinated phenols, and petroleum hydrocarbons from contaminated soils. Removals of greater than 90% were observed with surfactant concentrations of 1.5% using a blend of two non-ionic surfactants. These research groups also carried out treatability studies to recover surfactant from surfactant-contaminant solutions and were unsuccessful. They concluded that recovery of the surfactant was absolutely necessary for cost efficiency. Kunze and Gee (1989) used Triton –X-100, a non-ionic surfactant, and CitriKleen to look at the recovery of PCBs from soil. The use of the non-ionic surfactant precluded easy separation of the surfactant from the contaminants for reuse. Jafvert *et. al.*, (1995) demonstrated that the enhanced water solubility of hexachlorobenzene is clearly a function of the surfactant chosen and proposed a micelle-water partition coefficient related to the octanol-water distribution of the pollutant.

Soil flushing and soil washing remain plagued to this day by the desire of the practitioners to create strong emulsions that do not allow for surfactant recovery. CH_2Mhill (1997) identified recovered water treatment as the most important research need facing surfactant flushing. Surfactant costs constitute the single largest cost in a surfactant-enhanced remediation, and decontamination of the surfactant stream is essential (Krebs-Yuill *et. al.*, 1995). Other problems of surfactant use include foaming and biological toxicity at the local wastewater treatment facility if wastewater from the Surfactant Enhanced Aquifer Remediation (SEAR) program is sewered at even 0.1% surfactant concentration (Britton and Dwarakanth, 2002). The problem of surfactant recovery had not been solved (Sabatini *et. al.*, 1999). Options for recovery or treatment of emulsified surfactants include sorption, ultrafiltration, chemical complexation, chemical transformation, foam fractionation, and biological treatment (Britton and Dwarakanth, 2002).

Reliable cost estimates (Table 1.1) for the various treatment options are very limited, since each project must be considered for the geology, pollution type and concentration, and whether the reported costs include site evaluation and

engineering. CH_2MHill (1997) compiled the values for the 26 case studies in their survey of surfactant-enhanced soil flushing projects. They expressed great caution in the use of the following values.

Table 1.1: Range of Costs for Surfactant Enhanced Soil Flushing (CH_2MHill, 1997)

	$MM/acre	$/cubic yard	$/gallon NAPL recovered
Low Cost Report	0.57	65	21
Median Cost	3.4	270	86
High Cost Report	7.5	750	239

Of particular interest to Hill AFB is the reported success of the micellar flood demonstration conducted at the Operating Unit (OU) 2 site. OU2 is a closed chemical disposal area located in alluvial sand aquifer and naturally confined by clay. Sodium dihexyl sulfosuccinate (8%), isopropyl alcohol (8%), and sodium chloride was the surfactant mixture of choice for the demonstration. Approximately 98% of the DNAPL was removed at an estimated cost of $3,000 per gallon as compared to the original estimate of $32,000 per gallon over a period of 30 years for conventional pump and treat (GWTRAC, 1998, case FLSH0019). In another report on the same site, foam was deliberately created to control migration of DNAPL and to focus the flow of the flush (Meinardus *et. al.*, 2002).

Site selection remains a key determinant of the potential success of any surfactant remediation program. Of the pilot or full-scale demonstrations surveyed by GWRTAC (1998), 43% of the projects were in hydraulically contained areas with an additional 28% in natural containment and 11% performed in test cells. Less than 5% of the respondents were actively remediating sites of depth greater than 50 feet. Of the respondents that reported hydraulic conductivity data, about ½ of the soils were in the range of 10^{-3} to 10^{-4} cm/sec while another 40% were in the range of 10^{-2} to 10^{-3} cm/sec.

Hill AFB has a long history of the use of innovative technologies for site remediation. Bedient *et. al.*, (1999) describe site preparation and monitoring techniques for nine field demonstrations performed at Hill AFB. Hill AFB is contaminated with jet fuel, PCBs, chlorinated benzene, benzene, tolulene, ethylbenzene and xylenes (BTEX), TCE, dichloroethylene (DCE), and dicloroacetate (DCA). The site materials consist of 0-19 M (9M) average sand and gravel overlaying up to 60 M silty clay infused with fine sand stringers and fatty clay inclusions. Hydroconductivity is high in the horizontal direction and low in the vertical direction. Test cells, of dimensions 3 M by 5 M, were constructed with metal barriers driven from the surface through the clay layer to a depth of about 5 M. Each cell was constructed with four upstream wells and three downstream recovery wells. Studies included pump and treat simulation with water only; followed by tracer addition of one non-partitioning and two partitioning molecules to determine hydrology and

organic chromatographic characteristics of the NAPL and soil. Details of the various tests are available (e.g., Brown *et. al.*, 1999, Knox *et. al.*, 1999).

However, the surfactants removed 90% of the LNAPL after seven pore volumes of surfactant were applied. At many sites, NAPL exists in the vadose zone as both free-product and as saturated soil or ganglia. The challenge is to mobilize both the free product and make the saturated soil release the organic. It is generally recognized, however, that it is critical to the control of any polluted groundwater to gain control over the source (Wilson and Clarke, 1994). Perhaps the first published report of surfactant flushing was a series of studies to optimize surfactants for the removal of gasoline from soil (Texas Research Institute, 1979, 1985). These authors used a blend of anionic and non-ionic surfactants. A series of later studies (Ellis *et. al.*, 1984 and Ellis *et. al.*, 1985) focused on the flushing of PCBs, chlorinated phenols, and petroleum hydrocarbons from contaminated soils. Removals of greater than 90% were observed with surfactant concentrations of 1.5% using a blend of two non-ionic surfactants. These research groups also carried out treatability studies to recover surfactant from surfactant-contaminant solutions and were unsuccessful. They concluded that recovery of the surfactant was absolutely necessary for cost efficiency. Kunze and Gee (1989) used Triton –X-100, a non-ionic surfactant, and CitriKleen to look at the recovery of PCBs from soil. The use of the non-ionic surfactant precluded easy separation of the surfactant from the contaminants for reuse. Soil flushing and soil washing remain plagued to this day by the desire of the practitioners to create strong emulsions that do not allow for surfactant recovery. Surfactant costs constitute the single largest cost in a surfactant-enhanced remediation, and decontamination of the surfactant stream is essential (Krebs-Yuill *et. el.*, 1995). The problem of surfactant recovery had not been solved by 1999 (Sabatini *et. al.*, 1999). One new surfactant technology has been proposed (Severin 2000, Severin and Nolan, 2002) that has potential to overcome some of the surfactant recovery issues. A proprietary blend of surfactants (Rhema Super Matrix, Rhema Products, Inc.) has been shown to mobilize PCBs from spiked lake sediment and reject and concentrate the PCBs into a sacrificial oil layer. The oil may be applied in the mixture of soil, oil, and surfactant, or the soil and surfactant may be contacted away from an independent oil/surfactant contact chamber.

The following discussion centers on some competing technologies that have been used on a number of soil contaminants, and may not necessarily be amenable for TCE treatment strategies. Numerous alternatives to pump and treat exist. Hayes *et. al.*, (1996) reviewed the technologies designed to control NAPL. These include containment methods such as freeze walls, grout barrier, hydraulic intervention, sheet pile, and slurry walls. Of more interest to this project are the treatment methods including biological treatment, chemical oxidation, heat or steam flushing, vapor extraction, and stabilization.

One alternative to surfactant flushing has been the adaptation of solvent flushing. This process has been recommended for *ex situ* soil washing. Most of the solvent extraction of soil has been performed in Europe, with little use in the US. One of the waterborne solvents is the Basic Extraction Sludge Treatment (B.E.S.T.^{TM}) technology that is based on the use of trimethylamine. This solvent is completely miscible in water below 65 F° and allows for removal of oils from sludge and the recovery of trimethylamine by distillation. Trimethylamine is flammable in the presence of oxygen and this has been somewhat problematic to the successful commercialization of the process. Reported treatment costs range from $100-$400 per ton (EPA, 1994). Another system has been proposed to treat Kuwaiti oil spills (Science, 2001). Soil is excavated, treated with kerosene to mobilize the weathered oil, extracted of excess kerosene, piled, then treated with air and water to start a biological treatment process. The Japan Petroleum Energy Center and the Kuwaiti Institute for Scientific Research are jointly developing the process. Several proposed processes are being developed based on extraction followed by solvent distillation. One invention uses alcohols (Plunkett, E.L., 1999); another uses organic amines (Heins and Nowak, 1997), while a third uses liquid propane (Moses, 1988).

Biological treatment is broadly named by various modifications on the general theme, being known as bioremediation, bioventing, biosparging, or intrinsic bioremediation. Biological treatment has been used extensively for treating petroleum products including volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), phenolics, ammonia, and cyanide. The process was initiated by Sun Chemical in 1972 to remediate light petroleum around storage tank spills. There have been hundreds of successful demonstrations in the intervening 30 years. The process is limited to low to residual concentrations of pollutant. There are several reasons for this including inability to manage co-metabolites, pH, electron acceptors and donors, oxidation reduction potential (ORP) of the media, and toxicity of the pollutant or metabolites. The process may be initiated using naturally occurring bacterial populations, or augmented with selected organisms delivered to the matrix. One problem that potentially occurs, and is quite worrisome, is the potential to plug the aquifer when the organism grows into media. Another problem is alteration of the ORP of the media by either the organism metabolism or the introduction of the electron acceptor (vis. oxygen). This can manifest itself as plugging by iron oxide formation, or liberation of arsenic and selenium. Lack of pH control can lead to plugging of the aquifer by calcium carbonate formation. Severe process control makes the process unpopular for heavy clean up, such as NAPL. Most success has occurred in soils that are medium to coarse sand, and weathered bedrock. Success is unlikely if the pore permeability is less than 10^{-4} cm/sec. To approach any remediation, it is recommended to dig all material in excess of 10,000 mg/kg. One favored alternative aimed at remediation of the vadose zone is called bioventing. Air is drawn through the soil column and the

bacteria in the soil respond by consuming the volatile organic as they pass up the soil column. Bioventing of BTEX and total petroleum hydrocarbons (TPH) has been performed at a number of Air Force test sites (EPA, 2000) with cost estimates ranging from $10-60/ cubic yard of material treated). In another test case, chlorinated hydrocarbons were passively vented at a cost of $100 per cubic yard treated (EPA, 2000). Hill AFB successfully treated high concentrations of jet fuel with sequential SVE followed by bioventing for an estimated cost of $120 per cubic yard (EPA, 1995). One favored alternative is called bio-sparging for treating the aqueous phase. Wells are drilled into the aquifer and air is periodically sparged into the well. One of the major drawbacks to bioremediation is the complex reliance on the groundwater hydrology. Hayes *et. al.*, (1996) estimate that the extra caution needed to study the groundwater will add 15-30 % to the engineering costs of any bioremediation project. Analysis of the nutrient conditions and confirmation of the test organism will add an additional $30,000 to $75,000 per site. Nutrient and electron acceptor (e.g., peroxide) will run $1000 per month for a 200 gpm injection cell. Monitoring costs are 25-50% higher than with other projects. Compared to pump and treat with carbon, however, the high initial costs of biological treatment are soon recouped. Brown *et. al.*, (1985) estimated that biological remediation of a 6700 kg xylene site would cost $180,000-$270,000 over 18 months, whereas pump and treat carbon would cost $470,000-$850,000 over 10 to 20 years.

Chemical oxidation treatment is broadly classified as a chemical reaction catalyzed with an oxidant. Sub-classes of the process are Fenton's reagents, peroxide injection, or ozonation (Hayes, 1997). Target compounds are VOCs, PAHs, phenol, selected metals, and conventional pollutants. More recently, permanganate has been tried at a few sites. Most success with chemical oxidation has occurred with treating aromatic compounds (benzene, toluene, PAHs, etc.) using peroxide or ozone, which readily oxidize double bonded carbon. The greatest technical drawback appears to be the potential for non-specific reactions and loss of oxidant with natural soil organic matter. A second potential problem is the oxidation of non-specific metals (e.g., iron) and the resulting plugging of the aquifer. *In situ* technology implementation requires an upstream injection well and a downstream interceptor well. Good cost figures for *in situ* chemical treatment are not presently available, however, the Gas Technology Institute performed pilot studies in *ex situ* pilot slurry reactors (Hayes *et. al.*, 1996, pp. 431) and conducted a cost scale-up (GRI, 1994). Due to the high cost of treatment chemicals, the studies were based on a sequenced chemical dose followed by biological treatment. The treatment period required for about 90% removal of PAH was 40 days for chemical oxidation followed by 20 days of biological polishing. Significant time was required to resettle the soil slurry. Cost estimates ranged from $205 to $275 per cubic yard of soil, depending on the amount of peroxide used (1-3% by weight).

Soil flushing has traditionally been based on hot water or steam extraction. The process is very common in the oil drilling business for the recovery of spent oil fields (Lake, 1989). Hayes *et. al.*, (1996) describe the application to manufactured gas plant soil remediation. The process is based on desorption of contaminants from the soil matrix. The typical installation involves multiple injection wells on the perimeter of the contaminated zone and a collection well at the center. Broad criteria for successful implementation include the following. The target compound must be in a free and undissolved state (NAPL) and is not applicable to low concentrations in groundwater. The free-phase material must have a viscosity that is significantly lowered by increased temperature. The aquifer must have a high hydraulic conductivity ($>10^{-3}$ cm/sec). Natural geological features must vertically confine the source. Large volumes of polluted groundwater are expected, so a connection to a wastewater treatment plant is required. The process is energy intensive and access to an inexpensive power supply is absolutely required. One application of the process is the CROW™ system (Contained Recovery of Oily Wastes, Johnson and Guffey, 1990). Historical cost data are not available because of limited field experience. The GRI cost model was applied to the technology (Hayes *et. al.*, 1996) and it is expected that an average site with several wells required will cost $100,000 for wells and monitoring, $400,000 for equipment, $40,000 per month for operation, and $50,000 per month for energy. Operation is expected to require 3-9 months. Four test cases of thermal desorption of soil, with contamination ranging from VOC to pesticides to grease and oil, were reviewed by the U.S. Environmental Protection Agency (EPA) (2000) with costs ranging from a low of $105 per cubic yard to $350 per cubic yard of soil treated.

Soil vapor extraction (SVE) has also been called soil venting. The process is based on disrupting the equilibrium concentration conditions between the target organic, soil, water, and air by sweeping the soil above the groundwater with fresh air. The process is very good for sparingly soluble compounds with high vapor pressure (e.g., BTEX and gasoline). Highly soluble compounds are not removed well. Sites should be chosen to have a free depth above groundwater of at least 10 feet. Rapidly oscillating groundwater table depths are problematic. Activated carbon, biofiltration, or catalytic incineration, depending on the target compounds usually follows the SVE in the process train. Permeability of the soil must be greater than 10^{-4} cm/sec. Generalized cost figures are not available due to the number of site-specific variables. Follow-on treatment from the SVE will cost as much or more than SVE. As typical for many of the gasoline spill sites treated with SVE, the initial vapor is highly concentrated and may be treated for the initial period with flame or catalytic incineration. In the intermediate period after implementation, biological filtration or similar process may be used to advantage. Carbon is most effective in the final stages of an SVE remediation since the vapor is low in concentration. One report of SVE treatment followed by a biofilter for treatment of BTEX and TPH vapors was

estimated to cost $97 to 550 per kg BTEX destroyed (EPA, 2000, Patrick AFB site case). Resin adsorption following SVE of petroleum vapors was estimated to cost $23 per kg HC recovered (EPA, 2000, Vandenberg AFB site case).

Stabilization is also known as immobilization, encapsulation, fixation, or solidification. Stabilization is a physical or chemical process that limits contaminant mobility in a solid matrix. Augers or injectors place chemical directly into the soil and the chemical reaction ensues, in a process similar to cement grouting. A wide range of small companies provides the stabilization process. Some of the preferred stabilizers are cement, lime, fly ash, silicates, clays, chemical precipitants, and organic binders. Hayes *et. al.*, (1996) provide summary information on the favored stabilizers and the target pollutant. Some of the factors affecting the success of the process are as follows. Soils with high moisture content may require excess chemical. Oil and grease retard many chemical reactions. Soil pH may affect which chemical to choose due to unwanted chemical reactivity. The main drawback to the process is that the additives must come in direct contact with the soil, requiring that a very large number (thousands) of auger holes be punched at each site. The Electrical Power Research Institute (EPRI) reviewed the costs of the stabilization process (EPRI 1991). Treatment costs range from $100/ ton for cement treatment, $40-65 per ton for lime, $30-100 per ton for silicates, and $70-110 per ton for organic binders. Some of the process vendors are Chemfix Technologies, Clackman OR; Geo-con, Inc., Hialeah, FL; Silicate Technology, Salema, CA; and Solidtech, Inc., Morganville, NJ.

The following table (Table 1.2) is a partial list of the companies active in the soil remediation business and provides innovative treatment solutions.

Table 1.2: Innovative Treatment Approaches for NAPL	
Surfactants	
SEAR	Intera Inc.
SEAR	URS Corporation
Oxidants	
General Oxidant (selected from many)	Envirox, LLC
Fenton	Geo-Cleanse International, Inc.
Permanganate	Geo-Cleanse International, Inc.
ISOTEC modified fenton	ISOTEC
BIOX modified fenton/biodegredation	BioManagement Services, Inc.
Bio Remediation	
Molasses Injection (Poster 36, session B3)	ARCADIS, Geraghty & Miller
Vegetable Oil Injection	PARSONS
General enhancement, oil, oxygen, molasses	Geo-Cleanse International, Inc.
Lactate Injection	North Wind Environmental
Lactate Injection	JRW Technologies

Table 1.2: Innovative Treatment Approaches for NAPL	
Chitin	JRW Technologies
Reductant	
Ferox (pneumatic injection of ZVI powder	ARS Technologies
Base Catalysis (EPA experimental, PCB exsitu)	Battelle
Thermal	
Steam Stripping	Steamtech Environmental Services (Denmark)
Steam Stripping	Integrated Water Resources, Inc.
Electrical Resistance Heating	Current Environmental Solutions, Inc.
Direct Element Heating	TeraTherm, Inc.
Electrical Resistance Heating	Thermal Remediation Services, Inc.
Exsitu Mechano-chemical dehalogenation	Environmental Decontamination Limited
Barrier Walls	
Steel Sheet Piling	Waterloo Barrier Inc.
Surfactants	
Permeable reactive barrier (iron filings)	Environmental Technologies, Inc.
Soil Washing	
Particle segregation (PCB, Pesticide)	URS

In summary, recent technology evaluations and site history at Hill AFB indicate that continued evaluation of surfactant enhanced remediation technologies can lead to favorable and cost effective techniques. Of particular importance is the issue of surfactant recovery (reuse) and the ease of cleaning pollutants from surfactant emulsions. Severin and Harris (2002) proposed that a blend of surfactants with high detergency and low emulsion potential would be useful for some types of organic pollutants in that wastewater generation could be minimized and surfactant reuse maximized. This project examines the technical potential of this idea for capture of TCE.

1.3 Scope

MBI International (MBI) and Utah State University (USU) proposed a Three-Phase Program leading to full-scale implementation of one or more novel remediation technologies. Phase I of the program (this report) was a one-year laboratory test of two treatment systems. The first project investigated the benefits of soil and groundwater amendment with a biological culture (granule culture system) compared to a matrix of physical-chemical amendments for the treatment of TCE. In the second project of Phase I research (this Task III report), a novel surfactant system capable of stripping oily pollutants (diesel fuel, PCBs, and potentially other heavy pollutants) from soil will be tested with the Hill AFB samples. The advantage of this surfactant system is the unique capacity of the surfactant to reject oils to a clear and separate layer, leading to potential for recycling of the surfactant.

The Specific Objectives of Task III: Separation of pollutants from soils by surfactant washing was outlined in the amended Statement of Work (SOW) of February 2002. The objective of this task was to demonstrate the applicability of the MBI surfactant for removal of TCE from soil by surfactant flooding. The work was to concentrate on samples from OU1, OU2, OU4, OU12. However, the site manager requested and delivered OU5 soil and water instead of OU4. The task was divided into three subtasks:

Equilibrium distribution in solution: The distribution of TCE between water phase and free-product phase will be determined in tap water or site ground water as a function of surfactant addition. Rhema Super Matrix surfactant will be compared against the commercial product presently used at OU2. Tests will be run at ambient lab temperature and at 50 ^{0}F.

Shake Vial Separation Equilibrium: Samples of OU1, OU2, OU5, and OU12 were collected from core drilling near the source area. Cores will be tested for TCE and other chlorinated solvents in each core sample. Samples will then be split into 25 g allotments and each allotment will be contacted with water or suitable dose of surfactant dose (nominally 0-10% surfactant by volume in a water matrix). Treated samples will be shaken, and the distribution of TCE among soil, surfactant, and free-product phase will be determined. Tests will be performed at ambient lab temperature and at 50^{0} F.

Surfactant Flooding Column Simulation: Soil columns will be set-up to demonstrate the ability to perform the surfactant flooding technology on a larger scale. These tests are commonly performed in small pressure columns of approximate dimensions 2-inch diameter by 5-inch depth. Recovery of TCE as a function of pore volumes of surfactant passed will be determined.

1.4 Audience/Stakeholders:

The Hill Air Force Base is regulated under CERCLA and RCRA provisions of Federal pollution legislation. The site is operating under a 100-year compliance schedule to mitigate the effects of TCE dispersion to the surrounding groundwater.

- Hill AFB Management Team.
- Personnel responsible for specific site assessments.
- MBI Management Team for new product identification.

1.5 Stakeholder/ End-User Issues:

This Task III project was designed to give alternatives to the presently used surfactant system based on aerosol and used on-site at OU2 by Intera, Inc. The proposed "oil-splitting" technology, or low-emulsivity detergent, Rhema Super Matrix is produced by Rhema Products, Inc., Dearborn Heights, MI.

MBI has a license to seek new markets and sell this product into the environmental marketplace. There is no other known stakeholder issue.

2.0 Methods, Assumptions and Procedures

2.1 Test Objectives

The experimental design followed closely to the objectives outlined in the work plan (Statement of Work). More specifically, the tests were designed to:

1. Propose a series of surfactants with potential for success.
2. Challenge the surfactants with soluble calcium to cull those with strong reactivity.
3. Optimize the remaining surfactants to carry TCE in soluble micro-emulsion phase.
4. Establish the optimum surfactant range for the remaining surfactants using site waters.
5. Remove TCE from spiked site soils (1000 mg/kg spike) in shake vial equilibrium tests.
6. Remove TCE from spiked site soils (1000 mg/kg spike) in flow-through column studies.

Table 2.1: Performance Objectives

Type of Performance Objective	Primary Criteria	Expected Performance Metric	Actual Performance
1. Qualitative	Select Initial Surfactants,	Based on selecting the presently used surfactant, a reference standard, a couple of commonly used non-ionics, and the target Rhema Matrix cleanser.	Surfactants are readily available and we were able to select a wide range of products for screening.
2. Qualitative	Reactivity of surfactants with soluble calcium	Some surfactants were expected to have an unhappy reaction with calcuim, leaving a scum that would not be expected to pass through a tight soil matrix.	A number of products had severe reaction to calcium and these reduced the field from six products to three.
3. Quantitative	Equilibrium concentration of TCE in microemulsion	Those products that produced carried a large amount of TCE in the water phase were accepted.	Aerosol, Rhema, and a blend of Rhema and SLS showed the most promise and were retained for testing.

Type of Performance Objective	Primary Criteria	Expected Performance Metric	Actual Performance
4. Quantitative	Test in Site waters for optimum surfactatant blend	Expected that all products that reached level 3 screen would be optimizable	Met expections
5. Quantitative	Test for removal of TCE from site soils	Expected that some products would perform well,	No product removed more than 15% of the TCE spike into a single equilibrium wash
6. Quantitative	Removal of TCE from spiked soil in flow-through column tests.	Expected high removals for some products, and poor performance from others.	Uniformly poor results were obtained with less than 2% recovery of TCE in multiple pore volumes of extractant.

2.2 Test Site/Facility Characteristics

Hill AFB and earlier military installations that occupied the base have operated industrial facilities related to military aircraft since the construction of the Ogden Air Depot in 1940. Many different aircraft, missile, and weapons systems have been maintained and tested in the course of continuous operation and expansion of the base since its inception. These operations and past practices for waste disposal have resulted in the contamination of the soils and groundwater at the base. This contamination has also migrated off-base in the groundwater.

Hill Air Force Base was put on the National Priorities List in 1987. According to the Federal Facility Agreement (FFA) between Hill AFB, EPA, and the Utah State Department of Environmental Quality (UDEQ), Hill AFB is committed to completion of ongoing CERCLA activities at Hill AFB and is actively seeking innovative technologies to reduce the cost of required remediation efforts across the base. Hill AFB overlies three aquifers of which two are productive drinking water sources and are used by Hill AFB and the surrounding communities. In addition, there are seeps and springs, and field drains along the hillside south and north of the base that intercept waste petroleum and cleaning solvents containing BTEX, PCE, TCE and other contaminants at numerous discharge points.

Hill AFB is located in northern Utah, approximately 25 miles north of Salt Lake City, 5 miles south of Ogden and adjacent to Interstate 5. The base occupies approximately 6,700 acres in Davis and Weber counties. Davis-Weber Canal and Interstate 15 bound the base on the west. State Route 193 bounds it on

the south, the Davis-Weber Canal on the north and private property on the east.

The innovative technologies to be investigated in this study are relevant to a number of sites that exist throughout Hill AFB. Free product pools and residually contaminated soil exist within areas of OU1, OU2, and significant off-site, dissolved chlorinated solvent plumes are associated with OU1, OU2, OU4, OU5, and OU9. In addition, residual PCBs may exist in surface soils at sites OU5 and OU6. The nature of soil and groundwater contamination found throughout Hill is not uncommon in the Air Force system. The technologies to be evaluated in this project may provide significantly less expensive options for plume containment and control, source treatment, and overall site management that are necessary to reduce the risk of past disposal practices to the receptor community. Table 2.2 summarizes the nature of past practices and resultant contamination at a number of Hill AFB sites, along with the innovative technologies to be evaluated in this project that could be used to reduce the cost and increase the effectiveness of remediation.

Table 2.2: Hill AFB sites for which the proposed technologies are applicable.

Site	History	Contaminants	Media	Applicable Technology
OU1	Landfills, Chemical Disposal Pits, Fire Training Area	LNAPL, Chlorinated Solvents	Soil, Groundwater	Surfactant Washing, Accelerated Dechlorination
OU2	Chemical Disposal Pit	DNAPL, Chlorinated Solvents	Free Product, Soil	Surfactant Washing
OU4	Landfill, Road Side Dumping	Chlorinated Solvents	Soil, Groundwater	Accelerated Dechlorination
OU5	Open Waste Disposal, Leaking UST	Chlorinated Solvents, PCBs	Groundwater, Soil	Accelerated Dechlorination, Surfactant Washing
OU6	Substation, Open Waste Disposal	Chlorinated Solvents, PCBs,	Groundwater, Soil	Surfactant Washing, Accelerated Dechlorination
OU9	Open Waste Disposal	Chlorinated Solvents	Groundwater	Accelerated Dechlorination

2.3 Initial Screening with Calcium: Methods

Soluble calcium in groundwater poses a particularly challenging environment for numerous surfactants. One of the first semi quantitative tests for detergents was the old "bath tub ring" test in which soaps were titrated with calcium. Soap scum was an indicator of the amount of soap. In principle, the

following screening test was intended to warn the practitioner of potential to plug the groundwater column due to calcium-detergent precipitation.

Calcium interference tests were performed in OU5 water at 70^0F. Surfactants at various concentrations were prepared in OU5 water. Ten mL of each surfactant was then contacted with 10 g TCE in 25 ml vials, and the relative heights of each phase were measured after about 3 days contact. Each test vial was then opened and 0.5 mL (5% nominal alcohol to water) isopropanol was added. Contents were then re-shaken and allowed to settle for an additional 3-4 days. The relative heights of each phase were measured again. Powdered calcium chloride (dihydrate) was then added to each vial (0.3 g/vial, or 2.25% nominal $CaCl_2$ per gram water) and the contents shaken and resettled (3-4 days). Mixtures that retained 2 phases, or a third phase that was not a precipitated surfactant, were deemed suitable for further study.

2.4 Surfactant Screening, Sodium Chloride & Isopropanol as Co-Solvents: Methods

Screening tests continued to identify suitable ranges of surfactant in Lansing tap water. In these tests, isopropanol was used as a co-solvent in the ratio of 1:1 and 2:1 mass units of surfactant to ispropanol. Sodium chloride was used as surfactant desensitizer. Aerosol, SLS, and Rhema were used as the test surfactants. All concentrations of surfactant were on an "as delivered" basis.

One of the goals of the initial screening was to determine if stable middle-phase (micro-emulsion) or cloudy emulsion could be established as an indication of superior TCE dissolution into the water phase. In all tests, 10 g TCE were contacted with 10 g of various mixtures of surfactant/water/isopropanol/salt solutions. The full heights of the materials in each test vial total about 35 mm. Tests were first performed at room temperature, and then the vials were re-shaken for tests at 10°C.

2.5 Optimization: Analysis of the Emulsion, Microemulsion Phases: Methods

Another goal was to determine the best aqueous surfactant/isopropanol/salt systems that offer maximum extraction capacity of TCE from water. This was done by either contacting 2 g of TCE with 20 g surfactant mixture or 10 g TCE with 10 g surfactant mixture. In all cases, the amount of TCE used was large enough to give saturated surfactant systems upon thorough mixing. The TCE-saturated surfactant mixtures were allowed to equilibrate for at least 48 hours before analysis.

Analysis of all TCE samples (standards and unknowns) was done using headspace gas chromatography (HSGC). HSGC is a technique for the concentration and analysis of volatile organic compounds that makes use of the equilibrium between the volatile components of a liquid or solid sample.

A headspace sample is prepared in a sealed vial containing the sample, a dilution solvent, a matrix modifier, and the surrounding gas phase or headspace. In this method, 2.5% Rhema is the dilution solvent, and sodium chloride is used as the matrix modifier.

Calibration Standards: Calibration standards were prepared by dissolving specific amounts of TCE in methanol in 25 mL volumetric flasks. Tetrachloroethylene or perchloroethylene (PCE) standard, used as an internal standard, was also prepared by dissolving 1 mL PCE (1.6 g) in 25 mL of MeOH. An aliquot (5μL) of each stock TCE solution was added to separate headspace sample vials containing 7 g NaCl, 10 mL 2.5% Rhema and 5 μL PCE stock solution to obtain TCE concentrations 0.02 mg/L to 50 mg/L. Table 2.3 shows the amounts of TCE taken and the concentrations obtained.

Table 2.3: Calibration of the GC for TCE analysis

mg TCE in 25mL MeOH	mL TCE/25 mL MeOH	mg TCE in 5uL taken	Conc. (mg/L) in HS Vial
1.25	0.00085	0.00025	0.02
2.5	0.0017	0.00050	0.05
3.75	0.0025	0.00073	0.07
5	0.0034	0.00010	0.10
12.5	0.0085	0.0025	0.25
25	0.020	0.0060	0.58
75	0.050	0.015	1.5
125	0.085	0.025	2.5
250	0.17	0.050	4.0
500	0.34	0.10	10
1250	0.85	0.25	25
2500	1.7	0.50	50

Unknown Samples: The surfactant/isopropanol/salt systems under investigation were prepared by mixing appropriate amounts (g) of surfactant, isopropanol, and salt in 20 mL screw cup vials. TCE (2 or 10 g) were thoroughly mixed with separate surfactant/isopropanol/salt mixtures (10 or 20 mL) by hand shaking for 1 – 3 minutes. These mixtures were allowed to equilibrate for at least 48 hours. Aliquots of TCE-saturated surfactant/isopropanol/salt mixtures (0.25 mL) that have equilibrated for 48 hours and 5 μL PCE were added to separate sealed headspace (HS) vials containing 7 g NaCl and 10 mL 2.5 % Rhema and analyzed by HSGC.
Headspace Gas Chromatographic (HSGC) Analysis: HS vials containing standards and unknowns were analyzed with a Varian Series 3600 gas

chromatograph equipped with a Varian Series 3600 Headspace Sampler, a capillary flame ionization detector (FID) with a ceramic tip, and a 105 m VOCOL capillary column (Supelco), 0.53 mm I.D., and 3.0 µm film thickness. Clear glass, 20 mL, flat bottom (23 x 75 mm) crimp top headspace vials with Teflon-coated septa were used for all samples. A Windows-based Turbichrome data acquisition software was used for data analysis. Table 2.4 below shows the Gas Chromatography (GC) and Autosampler conditions used for the analyses.

Table 2.4: GC and Autosampler Conditions used for the Analysis of TCE.

Recommended Headspace Autosampler Conditions.	Recommended Headspace GC Conditions
• Platen Temperature: 90 ºC • Platen Equilibrium time: 3 min • Vial Equilibrium: 2 min • Vial size: 20 mL • Mixer: 1.0 • Mixer power: 3 • Stabilize: 1.0 min • Pressure time: 0.30 min • Pressure Equilibrium: 0.25 min • Loop fill time: 0.30 min • Loop Equilibrium: 0.20 min • Injection time: 1.0 min • Loop Temperature: 150 ºC • Transfer Line Temperature: 180 ºC • Extractions per vial: 1 • GC Cycle time: 25 min • Carrier Gas Pressure: 10 psi • Vial Pressure: 10 psi	• Initial column temperature: 45 ºC • Initial hold time: 0 min • Prgm 1: Final column temp: 62 ºC • Prgm 1: Column Rate in %/min: 10 • Prgm 1: Column hold time: 0 min • Prgm 2: Final Column temp: 220 ºC • Prgm 2: Column Rate in %/min: 20 • Prgm 2: Column hold time: 1min • Injector temperature: 190 ºC • Detector temperature: 250 ºC • FID A Initial attenuation: 16 • FID A Initial Range: 10 • Method Complete time: 10.60 min

Calibration Plot: Standard TCE solutions (5 µL) containing 5 µL PCE were analyzed and the peak areas recorded and plotted against the amount of TCE injected (mg), equivalent to the amount (mg) of TCE in the HS vial, Figure 2.1. To verify the consistency of the amount of sample injected, a plot of the amount of TCE injected versus the area ratio of TCE to PCE (A_{TCE}/A_{PCE}) was generated, Figure 2.2.

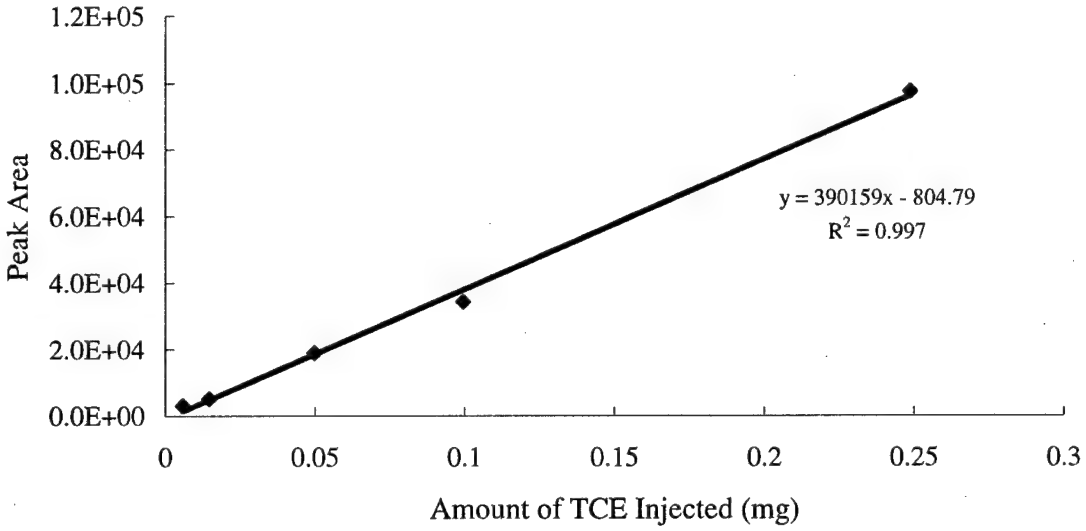


Figure 2.1: Calibration Plot for TCE in 2.5 % Rhema – Amount vs. Peak Area

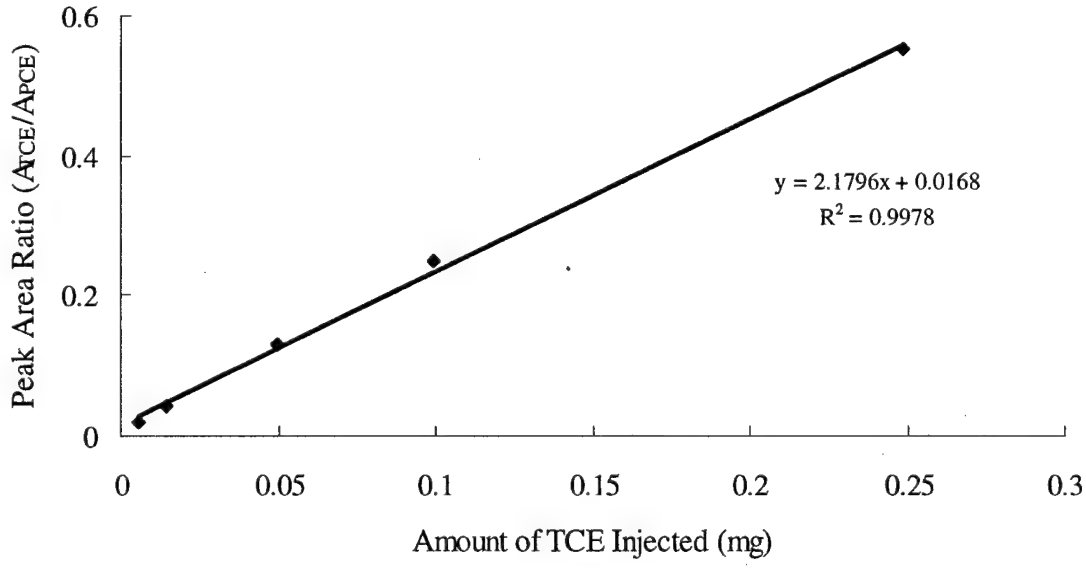


Figure 2.2: Calibration Plot for TCE in 2.5 % Rhema – Amount vs. Peak Area Ratio

A linear relationship obtained between amount injected and ratio of peak area (Figure 2.2) indicated that there was consistency in amount of sample

injected. Unknown TCE was therefore calculated from the equation of the relationship between the amount injected and the TCE peak area.

2.6 Optimization in Utah Site Waters OU1, OU2, OU5, and OU12: Methods

The evaluation of the surfactants Rhema, aerosol, and blended Rhema:SLS in the Utah waters was performed by selecting a range of salt and surfactant concentrations based on the optimum conditions established with Lansing Tap Water at 10°C. The matrices tested in the Utah water at 10° C were:

- 1:1 Rhema:isopropanol with 3, 4, and 5% surfactant and a range of 2, 3, and 4% sodium chloride.
- 2:1 Aerosol:isopropanol with 3, 4, and 5% surfactant and a range of 0.5, 1, and 1.5% sodium chloride.
- 1:1 Rhema:SLS:ispropanol with the surfactant blend based on 6:1 Rhema:SLS and having a total surfactant concentrations of 4, 5, and 6% and a range of 2, 3, and 4% sodium chloride.

The amount of TCE in the surfactant mixtures was determined by headspace GC using perchloroethylene (PCE) as internal standard. Phase diagrams showing the amount of TCE (mg/mL), Salt (%), and surfactant (%) were plotted and optimum surfactant mixtures determined and used for column extractions of TCE contaminated Utah soils.

TCE calibration plots were generated from standard TCE and PCE solutions as previously described in the previous section monthly report using headspace GC. TCE from Utah water samples was calculated from peak areas using the equation of the line.

Utah Water Samples:
Rhema, aerosol, and Rhema/SLS blend solutions (20 mL each) containing different amounts (%) of surfactant, isopropanol, and salt were contacted with 2 g TCE and allowed to equilibrate for 48 hours at 10 ºC. Various amounts of emulsions and microemulsions were formed and their characteristics and behavior under different conditions are described below.

2.7 Shake Vial Tests: Methods

This work focused on the recovery of TCE from spiked soil samples using three surfactant solutions developed earlier in this project. The test soils were OU1, OU2, OU5, and OU12 spiked with 1,000 mg/kg TCE. The three surfactant solutions were prepared in the waters from each site based on previously conducted optimization studies. After preparation, the surfactant samples were chilled to 50°F and filtered to remove precipitated material that formed from contact with the amendments and the site waters.

- Aerosol (2:1) ratio surfactant:isopropanol, 0.5% salt with all waters
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 3% salt in OU1, OU2, OU12
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 4% salt in OU5
- Rhema/SLS (6:1) with (2:1) ratio surfactant:isopropanol, 3% salt in all waters

Tests were performed with two similar methods. Results from the first method led us to suspect that volatilization of TCE might have been a problem in the procedure, so the tests were repeated with Method 2 designed to reduce the potential for volatile losses. Results from Method 2 were similar to Method 1, so both sets are presented.

Recovery of TCE from the soils varied marginally by the surfactant used, with Rhema Super Matirx giving slightly higher results. Overall, recovery was between 8 and 15% of the total initial TCE spike.

Method 1: Soil (10 g) was placed into a screw cap vial. TCE in the amount of 6.8 µL (9.9 mg) was added to the soil sample and the screw cap was closed. The vial was shaken to disperse the TCE. Surfactant (15 mL) was then added to the vial and the contents reshaken. The vial was then placed into a 50°F chamber for a few days. A 10 mL volume of surfactant was recovered and placed with 7 g salt into a GC headspace vial. The headspace vial was then capped and the contents analyzed. All samples were run in triplicate.

Method 2: A plastic sheet was used to construct a confined atmospheric space. A beaker of TCE was placed into the confined atmosphere and the atmosphere was allowed to equilibrate with the TCE. All further manipulations were performed within the saturated atmosphere. Two sets of samples were prepared, spiked samples and atmospheric controls. The spiked samples of soil (10 g) were pepared as follows in GC head space vials. Soil was placed into the vial and TCE in the amount of 7 µL (10.2 mg) was added. The vial was shaken to disperse the TCE. Surfactant (29-32 mL) was then added to completely fill the vial to eliminate headspace. The vial was capped and crimped, and the contents reshaken. After the contents of the vial settled, a 10 mL volume of surfactant was recovered and placed with 7 g salt into a GC headspace vial. The headspace vial was then capped and the contents analyzed. Controls were prepared by the same method except that no additional spike was added to the soil. Recovery of TCE from the controls represented the small amount of TCE that was potentially adsorbed from contact with atmospheric TCE vapor. All samples were run in triplicate.

2.8 Column Construction: Methods

The soil column design was finalized as depicted in Figure 2.3. The apparatus consisted of a 2 L separatory funnel used as a surfactant reservoir

suspended above the column. The flow inlet to the column was located at the base of the column. A full-port cylinder valve controlled the flow. The details of the column internals are presented in the next paragraph. The exit from the column is at the top of the unit. A flow stop valve is located just prior to the discharge line. The discharge line flowed into a 2 L separatory funnel used as the collection vessel for spent surfactant. The available head in the system was between 90 and 100 cm water, representing the high and low fluid levels in the inlet reservoir. During hydraulic capacity testing, flow was maintained constant by periodically replacing fluid in the reservoir at some mid-point between high and low volume. The target fluctuation in the reservoir level was less than 5 cm. During tests with surfactant and contaminated soil, the reservoir was filled with 1.5 L surfactant and allowed to fully drain through the column such that about 8 volume displacements were achieved through the soil. All tests were run in a controlled environmental chamber set at 10°C to simulate groundwater temperature conditions.

Each column consists of a piece of 5 cm diameter by 10 cm tall glass pipe. Compression fittings are used to hold milled Teflon plates on the top and bottom of the column. The internal structure of the column, from bottom to top consists of a piece of polypropylene weave cloth rested on the Teflon

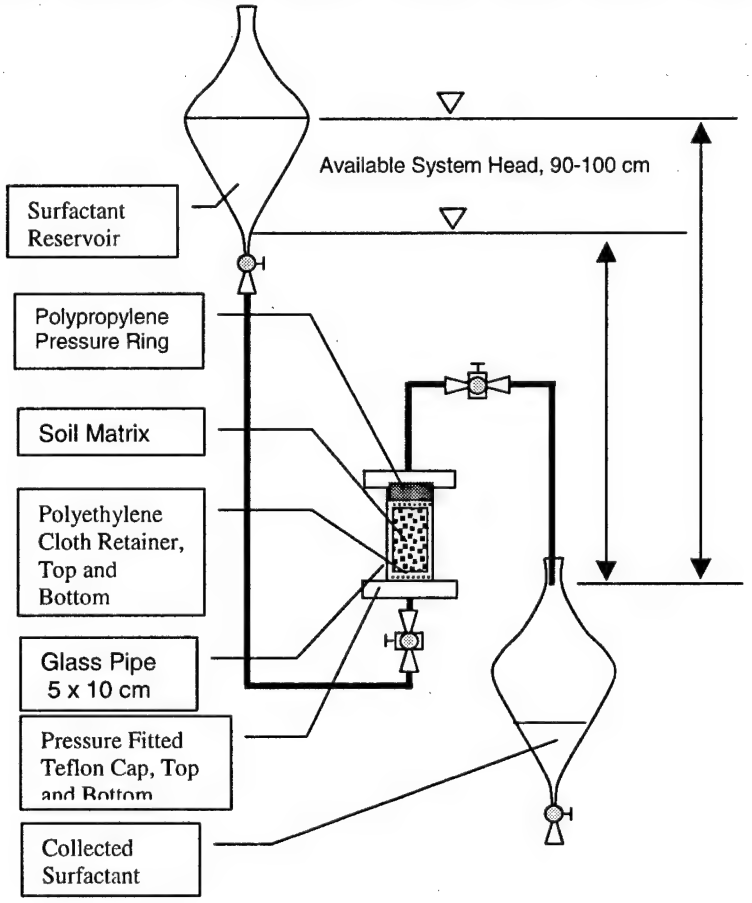


Figure 2.3: *Schematic of Gravity Soil Column Design*

bottom to keep solids from entering the entrance piping. Approximately 300 g of test soil is charged into the reactor. The test soil is packed tightly to remove as many air spaces as possible. A second piece of polypropylene weave cloth is placed on top of the soil sample. A 2 cm tall slice of 2-inch polypropylene drainage pipe is then placed on top of the cloth as a compression fitting. As the Teflon top is fitted onto the glass pipe, the compression fitting presses the cloth and compresses the soil sample. This keeps the soil from fluidizing during the test and minimizes the formation of unwanted air spaces within the column.

2.9 Hydraulic Analysis of Soil Columns: Methods

Hydraulic analysis of the columns was performed using a modified Darcy equation for flow through a porous medium (Freeze and Cherry, 1979, Severin and Grethlein, 1996). The flow equation follows a series resistance law for steady state flow in which the system losses (column, tubing, piping, etc without packed soil) are analyzed and subtracted independently from the total losses (column and piping plus packed soil). The parameters are presented in Table 2.5.

Table 2.5: Nomenclature for the Darcy Equation

	Units	Description		Units	Description
μ	dyne cm^{-2} sec^{-1}	Fluid viscosity	L	cm	soil depth
A	cm^2	Column area	*l*	cm	virtual depth of apparatus
g	cm sec^{-2}	Gravitational constant	P	dyne cm^{-2}	available pressure
h	cm	Available water (pressure head)	Q	cm^3 sec^{-1}	steady state flow
K	cm sec^{-1}	Soil conductivity permeability	R	cm^{-2}	soil resistance coefficient
k	cm sec^{-1}	Virtual system conductivity permeability	r	cm^{-2}	system resistance coefficient

Equation 1 is the Darcy Equation written for series resistance where the flow, Q, is proportional to the available pressure, P, divided by the sum of the resistances. In a strictly gravity flow condition, the available pressure is proportional to the fluid height in the system, h.

$$Q = \frac{PA}{R\mu L + \mu l} = \frac{\rho g h A}{R\mu L + r\mu l} \qquad (1)$$

Defining the soil conductivity, K, and the virtual conductivity of the system piping in terms of the resistance coefficient, viscosity, gravitational constant, and viscosity yields equations 2 and 3.

$$K = \frac{\rho g}{\mu R} \tag{2}$$

$$k = \frac{\rho g}{\mu r} \tag{3}$$

The ratio of the flow to the available pressure head is proportional to the inverse of the sum of the reciprocal conductivity. The value *l/k,* represents the resistance inherent within the system in absence of the soil matrix. This value can be measured independently. The soil conductivity, K (cm/sec) may then be back calculated from knowledge of the depth of the soil column, L.

$$\frac{Q}{hA} = \left(\frac{L}{K} + \frac{l}{k}\right) \tag{4}$$

2.10 Column Extraction Tests: Methods

Column extraction tests were performed using two series of tests. In the first series (Method 1), soil was dosed with 1,000 mg/kg TCE and left in capped glass jars for 5-6 weeks prior to testing. Columns were loaded with about 300 g of TCE spiked soil. All soils and solutions were brought to constant temperature (50° F) overnight before testing. Some of the test waters reacted with the salt:surfactant:alcohol solution to produce precipitates. No attempt was made to alter the condition of the surfactant solutions. Column extractions were performed at 50° F in a temperature controlled room. A 250 ml salt pretreatment was followed by surfactant treatment. Flow was by gravity drainage. Pretreatment fluid and surfactant were collected from the column into open flasks. Samples were collected for analysis after the salt flush and then periodically during the surfactant run. Some tests had limited percolation of surfactant and not all of the full 1 L dose of surfactant could be run through some of the columns. All columns were stopped after 16 hours drainage time. Tests that had limited percolation of surfactant are labeled in bold. These are indicated for any test that passed less than 150 ml of the 250 ml presalt solution, and less than 900 ml of the 1,000 ml surfactant solution.

Low recoveries of TCE brought a concern that performing the test procedures at atmospheric conditions limited the recovery of TCE in the surfactant, probably due to evaporation. In Method 2, a plastic sheet was used to construct a limited exposure atmosphere. A beaker of TCE was placed within the plastic enclosure and allowed to equilibrate to create a TCE-saturated atmosphere and all soils were spiked in the controlled atmosphere chamber after equilibrating for at least five hours. All soils and solutions were brought to temperature (50° F) overnight before loading into the columns for extraction. Some of the site waters reacted with some of the

surfactant:salt:alcohol solutions to form precipitates. Precipitates were filtered from those waters on a No.1 Wattman filter prior to application of the fluid to the columns. Column extractions were performed in a 50° F controlled temperature room. A 250 ml salt pretreatment preceded each surfactant treatment. Flow was by gravity drainage. Pretreatment fluid and surfactant were collected separately from the column into 1.2 L Tedlar bags to limit volatile losses. Salt prewash and surfactant were analyzed separately. Results for Method 2 are presented in Table 2. Tests that had limited percolation of surfactant are labeled in bold. These are indicated for any test that passed less than 150 mL of the 250 mL presalt solution and less than 900 mL of the 1000 mL surfactant solution originally applied.

In both series of tests, water from each site was prepared with a pre-chosen concentration of surfactant, salt, and isopropanol. These were the same as used in the shake vial tests, with the exception that by the second series of tests, we had used all the available OU5 water. The second set of OU5 column tests was prepared using Lansing Michigan tap water.

oil. The composition is 47% benzene, 1,1-oxybis, tetrapropylene sulfonate sodium salt and 1% sodium sulfate in water.

Witconol:
Witconol SN-90 (Crompton Corporation, Greenwich, CT, 06831) is a viscous whitish suspension that is soluble in water up to about 20%. Higher concentrations form water gels. It forms very stable emulsions in the presence of oil that are difficult to break. Witconol is a non-ionic surfactant with a chemical composition of 100% C_{10}-C_{14} ethoxylated alcohols.

Triton:
Triton, a colorless liquid, has good solubility in water up to about 20% in solution. Higher concentrations in water are achievable with difficulty. A higher concentration of surfactant in water form viscous gels and granular solids that are unsuitable for solution chemistry. In the presence of oil, Triton forms a stable oleic emulsion and dissolves oil into the water phase. Relatively large amounts of oil are observed in the aqueous phase. Triton is a non-ionic surfactant with the chemical composition, polyoxyethylene (10) iso-octylphenyl ether (Triton X150).

Sodium Lauryl Sulfate (SLS):
SLS, or sodium dodecyl sulfate (Fisher Scientific, Fair Lawn, NJ 07410) is a white powder that is readily soluble in water up to 25% but has extremely low solubility thereafter. It forms an unstable emulsion in water but solubilizes oil to a large extent into clear waterborne micelles. SLS is an anionic surfactant.

Aerosol:
Aerosol MA80I has been used for full-scale soil flushing at Hill AFB by INTERA, Inc. and was recommended as a reference for this project. The surfactant is 78-80% active ingredient, sodium di(1,3 dimethylbutyl) sulfosuccinate in 5% isopropanol and water. The material is manufactured by Cytec Industries, Inc., W. Paterson, NJ.

Water and soil samples from the site were analyzed for gross chemical constituency. The Michigan State University Crop and Soil Science Laboratory, East Lansing, MI analyzed the soils. Results are presented in Table 3.1. Merit Labs of East Lansing, MI analyzed the water. Water samples had degraded somewhat by the time they were used in these tests. It is noteworthy that all samples had precipitated what appeared to be calcium and iron bearing particles. The OU2 sample looked peculiar, having a very low pH (range of 4-4.1) and of course, no detectable alkalinity at this pH. This sample was repeated three times to be sure of the analysis. Most likely, the high iron content could account for acid production as the sample oxidized and precipitated. No further analysis or speculation was made as to the cause of this phenomenon. Water results are presented in Table 3.2.

Table 3.1: Chemical Analysis of Soils from Hill AFB				
	OU1	**OU2**	**OU5**	**OU12**
pH	8.7 pH	8.8 pH	8.6 pH	8.8 pH
Phosphorus	14ppm	13 ppm	9 ppm	3 ppm
Potassium	1%	1%	1%	1%
Calcium	91%	65%	84%	89%
Magnesium	8%	34%	15%	11%
Organic Matter	<0.1%	0.60%	0.70%	0.10%
Michigan State University Soil and Plant Nutrient Laboratory Crop & Soil Science Department, East Lansing, MI				

Table 3.2: Analysis of Hill AFB Water Samples						
	OU1	**OU2**	**OU2**	**OU2**	**OU5**	**OU12**
pH	7.02 pH	4.08 pH	4.15 pH	4.08 pH	8.06 pH	7.27 pH
Alkalinity*	482 ppm	not detected	not detected	not detected	318 ppm	306 ppm
Chloride	30	126	136	133	131	49
Calcium	161	106	103	82	86.3	89.1
Iron	1.04	2.92	2.51	3.94	0.09	0.68
Magnesium	53.7	50.7	51.4	40.4	50.2	24.8
Sodium	70.1	115	118	158	65.3	43.5
Merit Labs, 1451 East Lansing Drive, East Lansing, MI						

* Alkalinity as calcium carbonate (Ca^{+2})

3.2 Initial Screening with Calcium

Soluble calcium in groundwater poses a particularly challenging environment for numerous surfactants. One of the first semi quantitative tests for detergents was the old "bath tub ring" test in which soaps were titrated with calcium. Soap scum was an indicator of the amount of soap. In principle, the following screening test was intended to warn the practitioner of potential to plug the groundwater column due to calcium-detergent precipitation.

Calcuim Interference phase tests were performed in OU5 water at 70°F. Surfactants at various concentrations were prepared in OU5 water. Ten mL of each surfactant was then contacted with 10 g TCE in 25 mL vials, and the relative heights of each phase were measured (Figures 4.1, 4.4, 4.7, 4.10, 4.13, 4.16) after about 3 days contact. Each test vial was then opened and 0.5 mL (5% nominal alcohol to water) isopropanol was added. Contents were then re-shaken and allowed to settle for an additional 3-4 days. The relative heights of each phase were measured again (Figures 4.2, 4.5, 4.8, 4.11, 4.14, 4.17). Powdered calcium chloride (dihydrate) was then added to each vial (0.3 g/vial, or 2.25% nominal $CaCl_2$ per gram water) and the contents shaken and resettled (3-4 days). Phase diagrams are presented in Figures 4.3, 4.6, 4.9, 4.12, 4.18).

Mixtures that retained two phases, or a third phase that was not a precipitated surfactant, were deemed suitable for further study. Table 3.3 shows the ranges of desirable performance, undesirable performance, and acceptable performance.

Table 3.3: Calcium Interference Tests in OU5 Water

Surfactant	OU5 Water	OU5 + Isopropanol	OU5+Isopropanol + Calcium chloride
Aerosol	Desirable, up to 20% surfactant	Desirable, up to 20% surfactant	Acceptable, but loss of TCE retention with increased calcium
Rhema	Acceptable up to 10% surfactant	Acceptable up to 25% surfactant	phase splits and precipitation
Dowfax	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant
Witconol	Unacceptable at all concentrations	Unacceptable at all concentrations	Unacceptable, all concentrations
SLS	Acceptable at all concentration	Acceptable at all concentration	Unacceptable at all concentrations, gel formation
Triton	Acceptable at all concentration	Acceptable at all concentration	Hazy Interface, no indication of strong TCE capacity
Surfactant	OU5 Water	OU5 + Isoprpanol	OU5+Isopropanol + Calcium chloride
Aerosol	Desirable, up to 20% surfactant	Desirable, up to 20% surfactant	Acceptable, but loss of TCE retention with increased calcium
Rhema	Acceptable up to 10% surfactant	Acceptable up to 25% surfactant	phase splits and precipitation
Dowfax	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant
Witconol	Unacceptable at all concentrations	Unacceptable at all concentrations	Unacceptable, all concentrations
SLS	Acceptable at all concentration	Acceptable at all concentration	Unacceptable at all concentrations, gel formation
Triton	Acceptable at all concentration	Acceptable at all concentration	Hazy Interface, no indication of strong TCE capacity

Aerosol initially showed a large solubilization of TCE into the water phase (Figure 3.1), and retained about the same degree of solubilization with addition of isopropanol (Figure 3.2). Upon addition of calcium carbonate, the water released the excess TCE (Figure 3.3). Rhema has acceptable behavior with doses up to 10% surfactant. Thereafter, the surfactant split into several complex phases (Figure 3.4). With added isopropanol, the complex phase splits were lessened and retarded until 25% surfactant (Figure 3.5). In the presence of heavy calcium brine, Rhema was completely unacceptable

with heavy precipitation within the TCE layer (Figure 3.6). Dowfax was acceptable up to 5% in OU5 water, but generated a precipitated surfactant layer at the TCE-water interface at higher concentrations (Figure 3.7). Addition of isopropanol resulted in increased precipitation (Figure 3.8), with slight improvement with added brine (Figure 3.9). Witconol was unacceptable at all concentration ranges in OU5 water, with three to four complex phases generated (Figure 3.10). Slight improvements were seen with isopropanol (Figure 3.11) and calcium additions (Figure 3.12). However, heavy precipitation of the surfactant rendered this surfactant completely unacceptable. SLS was acceptable at all concentrations up to 20% in water (Figure 3.13) and isopropanol (Figure 3.14), but was susceptible to precipitation and gel formation in the TCE layer with added calcium (Figure 3.15). Triton formed a hazy intermediate layer amounting to about 4% of the sample volumes (Figures 3.16, 3.17, 3.18). Based on this screening, we recommend elimination of Dowfax, Witconol and Triton from further testing. SLS will be used with limited brine, and Rhema will be used in concentrations less than 10%.

Figure 3.1

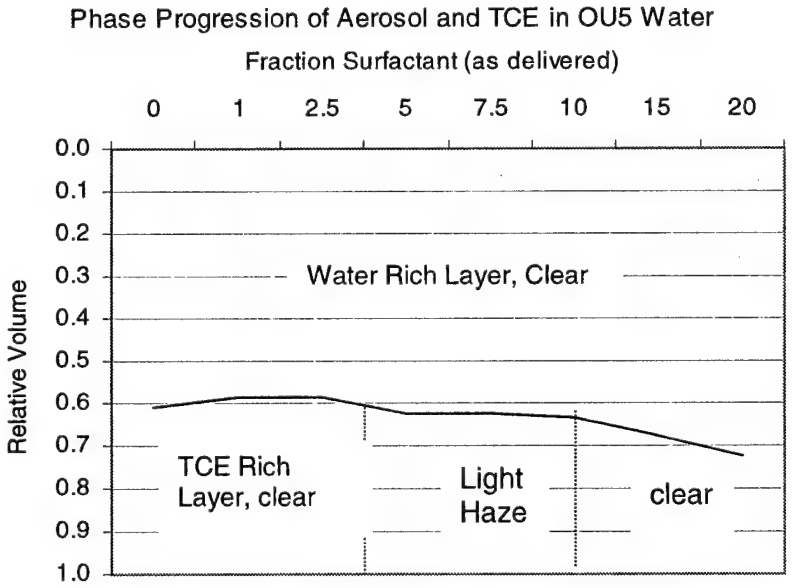


Figure 3.2:

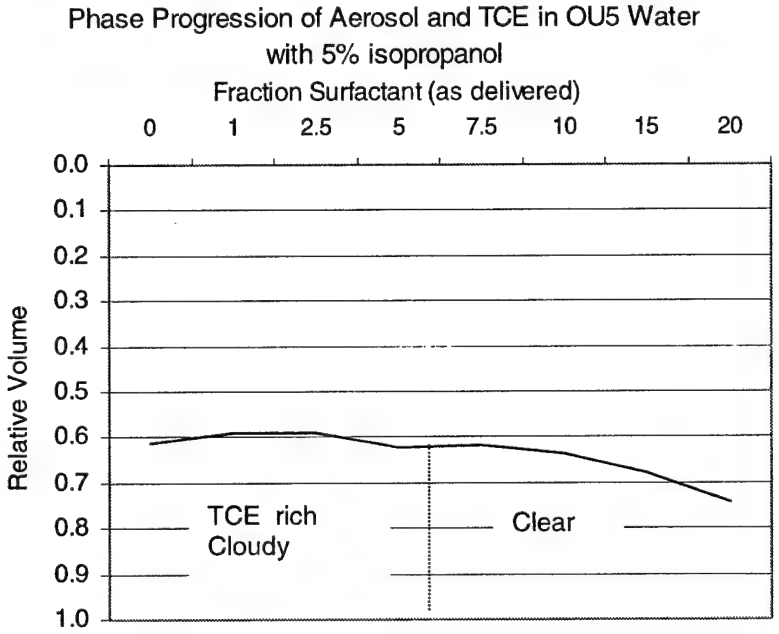


Figure 3.3:

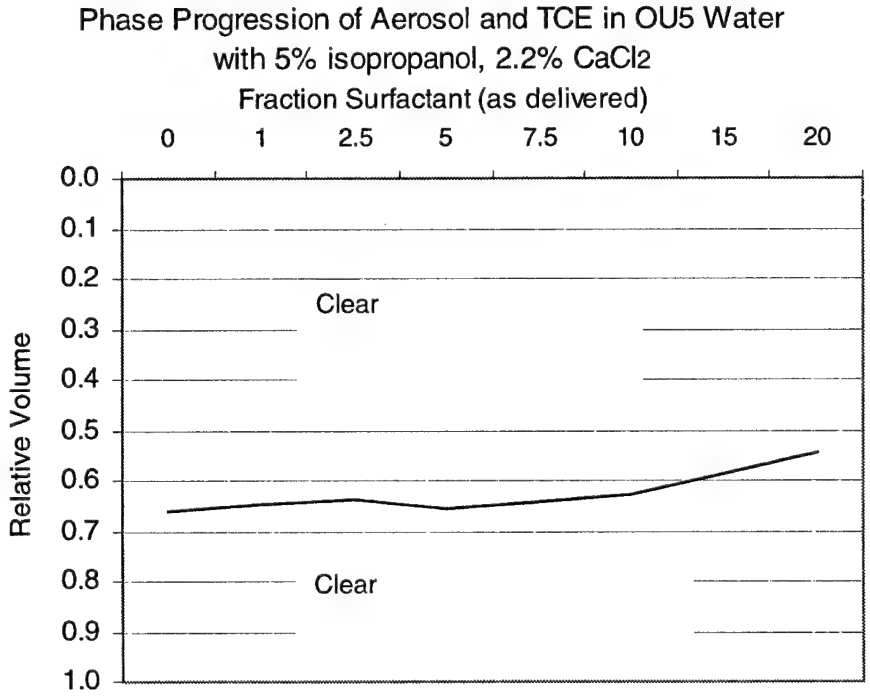


Figure 3.4:

Phase Progression of Rhema and TCE in OU5 Water

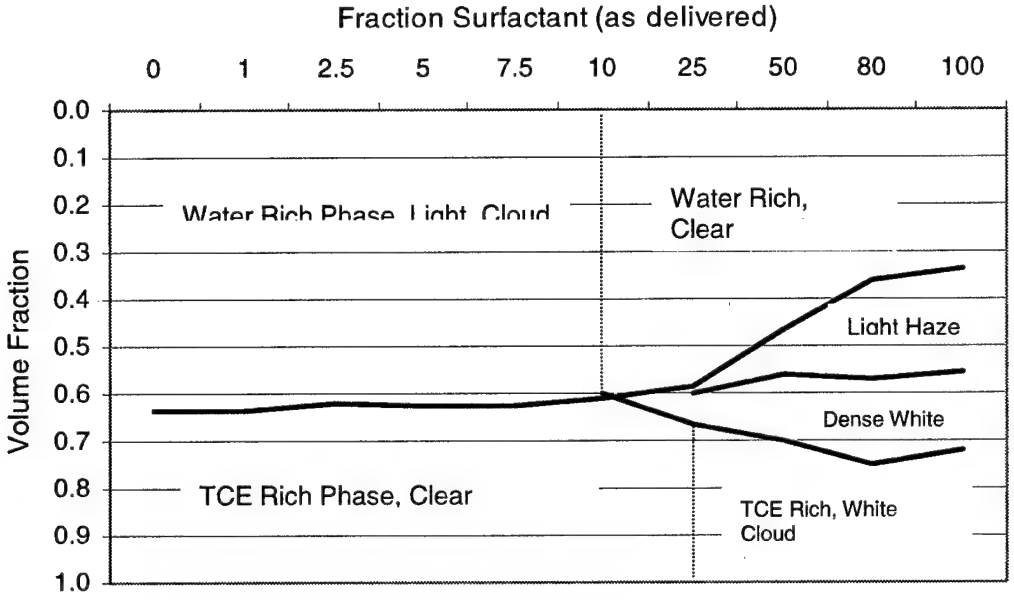


Figure 3.5:

Phase Progression of Rhema and TCE in OU5 Water, with Isopropyl Alcohol, 5%

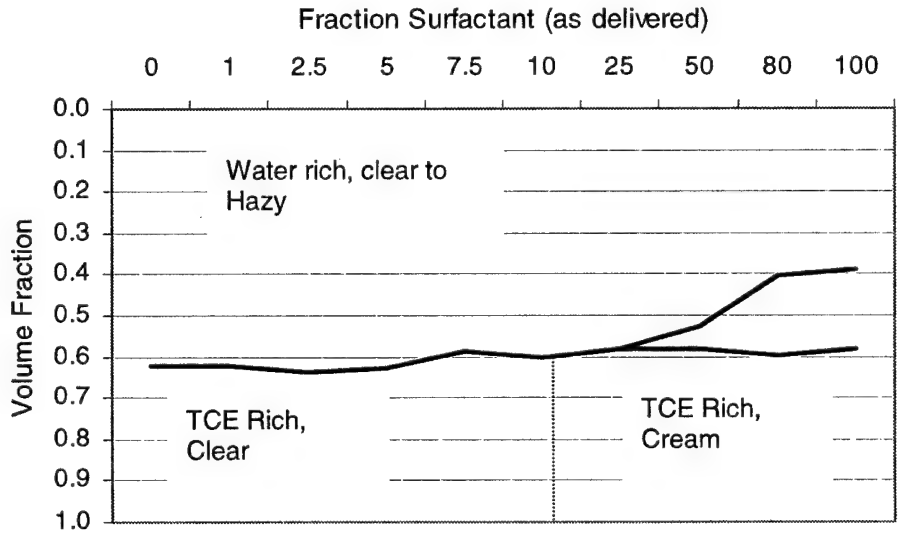


Figure 3.6:

Phase Progression of Rhema and TCE in OU5 Water, with Isopropyl Alcohol, 5%: $CaCl_2$ = 2.25%

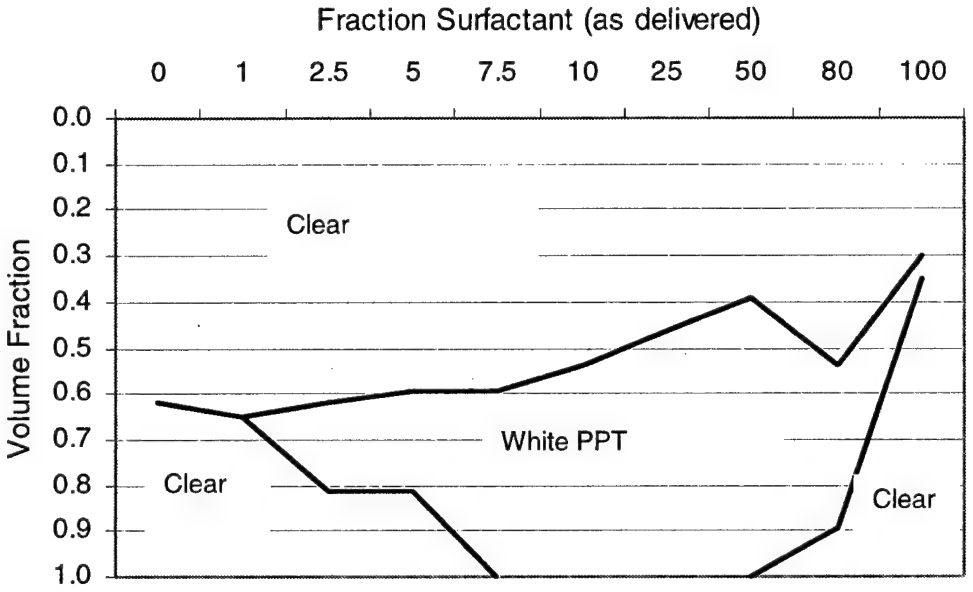


Figure 3.7:

Phase Progression of Dowfax and TCE in OU5 Water

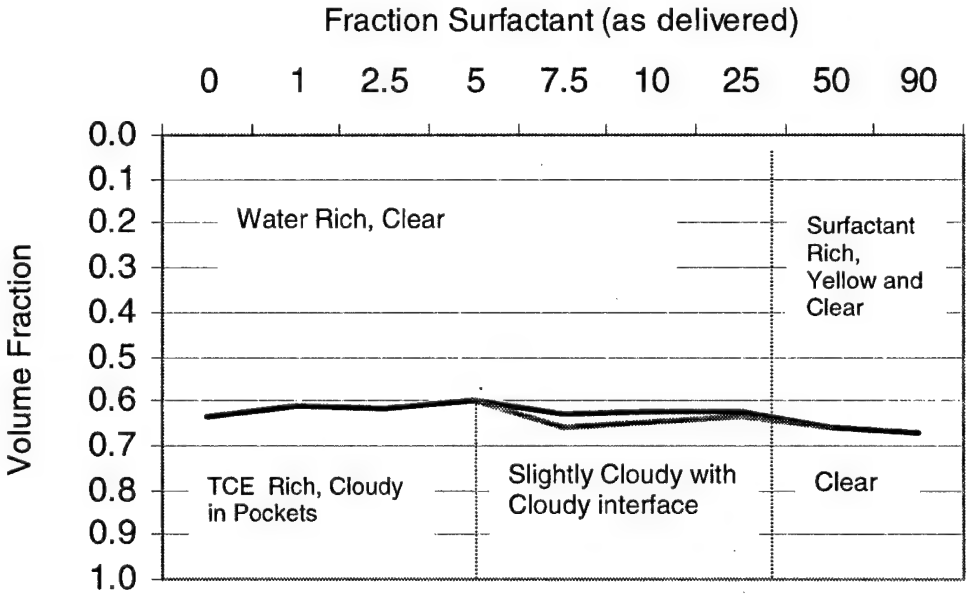


Figure 3.8:

Phase Progression of Dowfax and TCE in OU5 Water with 0.5 ml Isopropanol
Fraction Surfactant (as delivered)

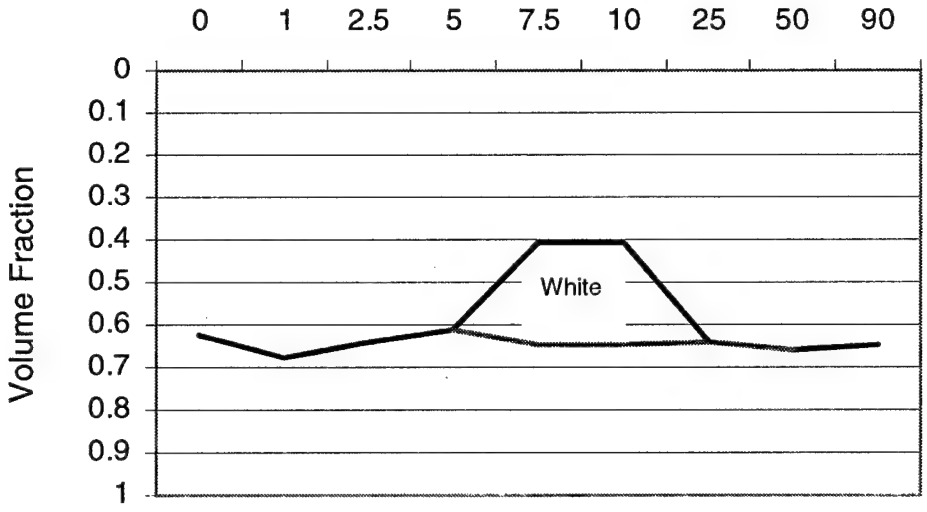


Figure 3.9:

Phase Progression of Dowfax and TCE in OU5 Water with 5% Isopropanol, $CaCl_2$ 2.25%
Fraction Surfactant (as delivered)

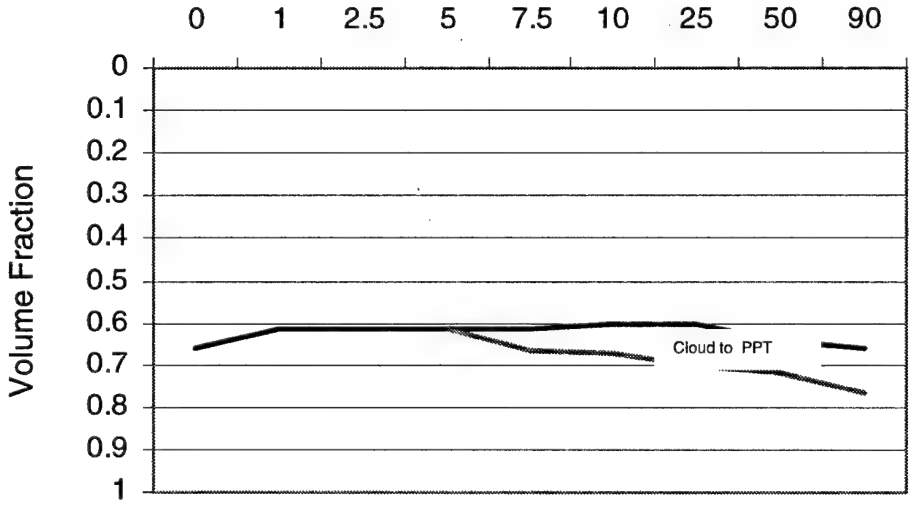


Figure 3.10:

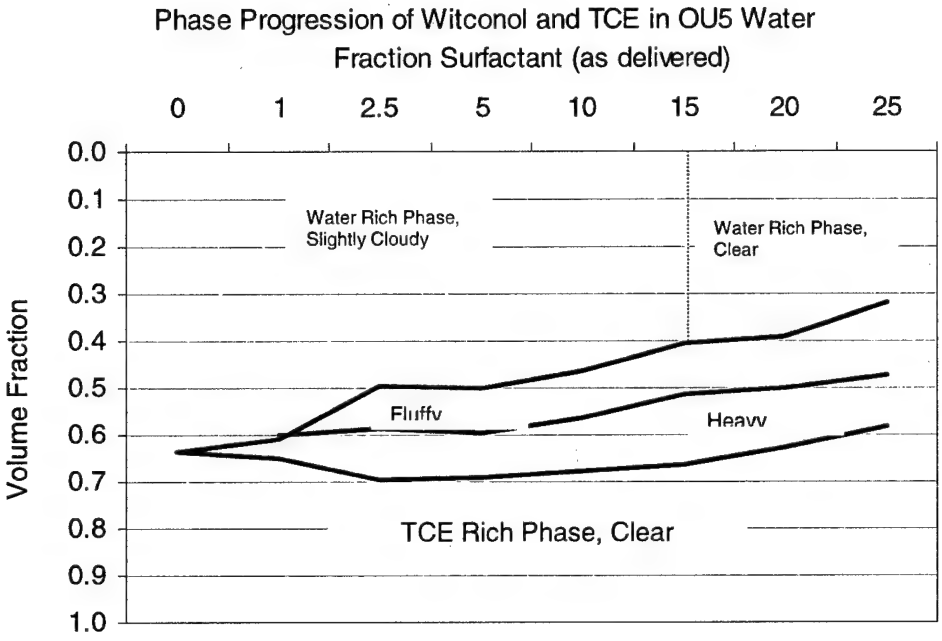


Figure 3.11:

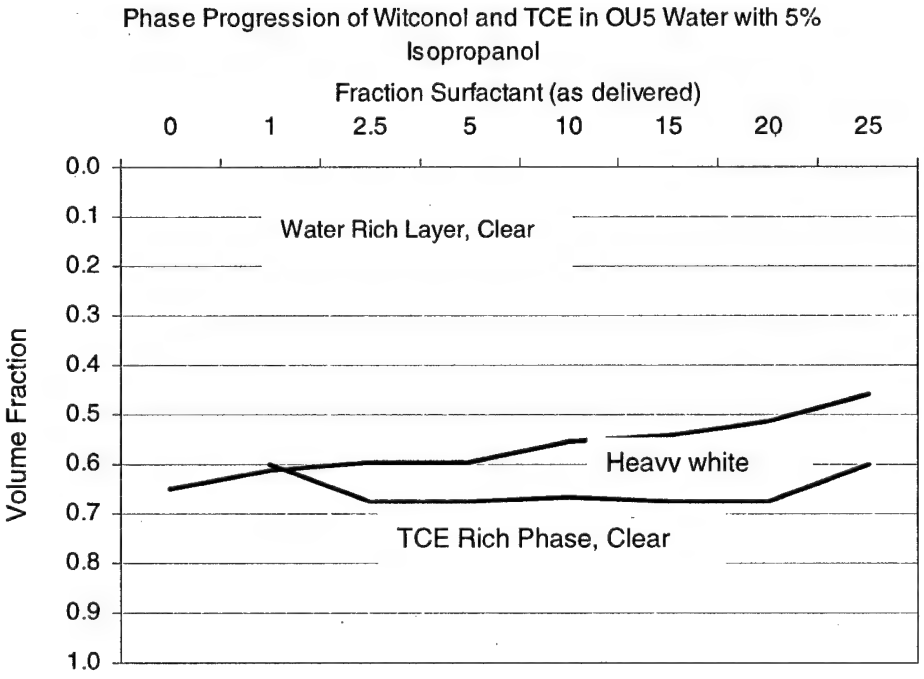


Figure 3.12

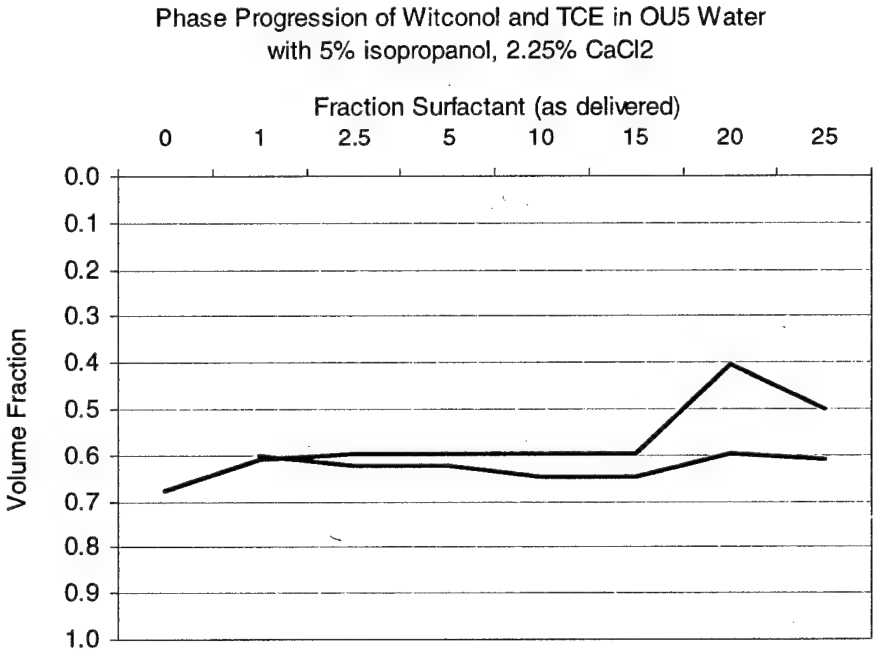


Figure 3.13:

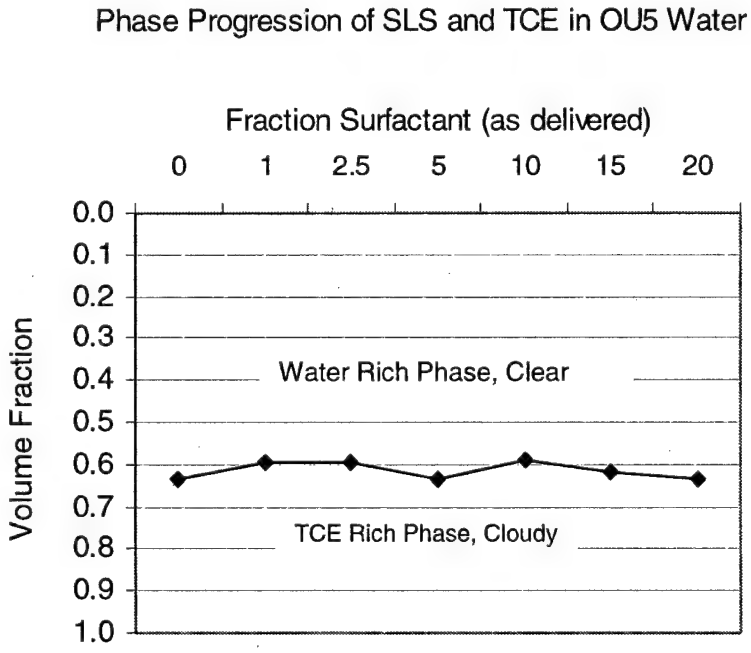


Figure 3.14:

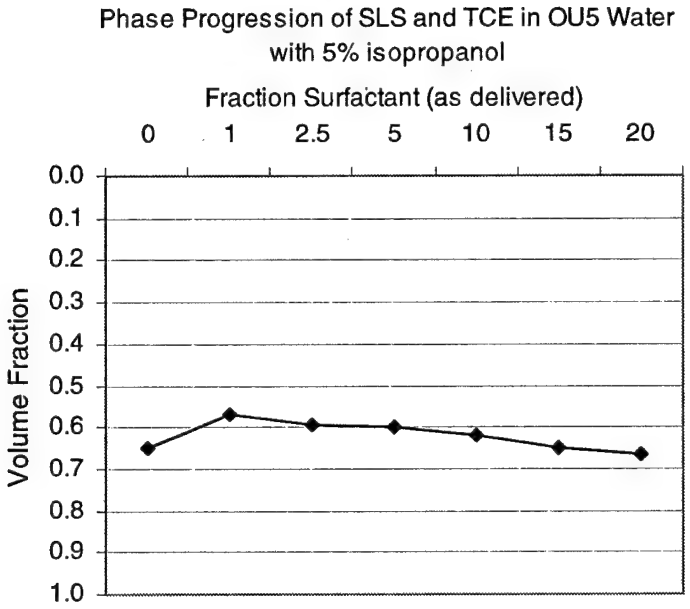


Figure 3.15:

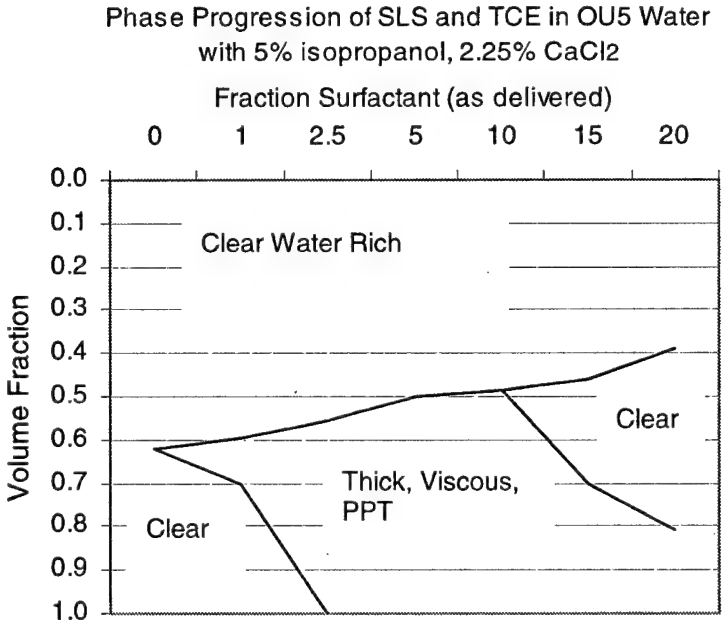


Figure 3.16:

Phase Progression Triton and TCE in OU5 Water

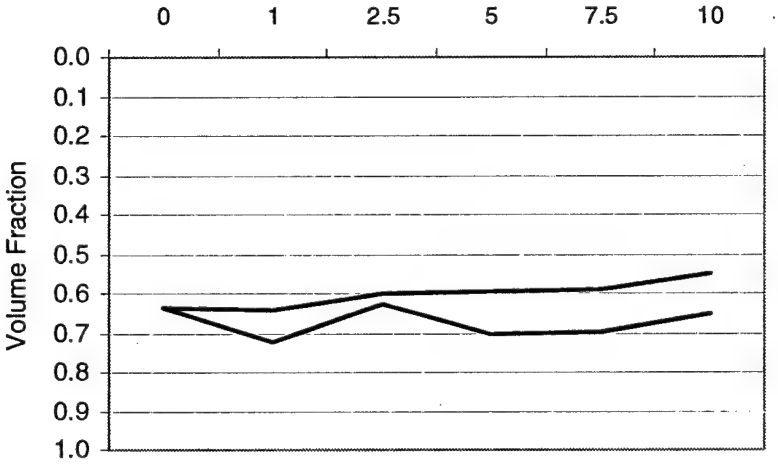


Figure 3.17:

Phase Progression Triton and TCE in OU5 Water with 5% Isoprpopanol

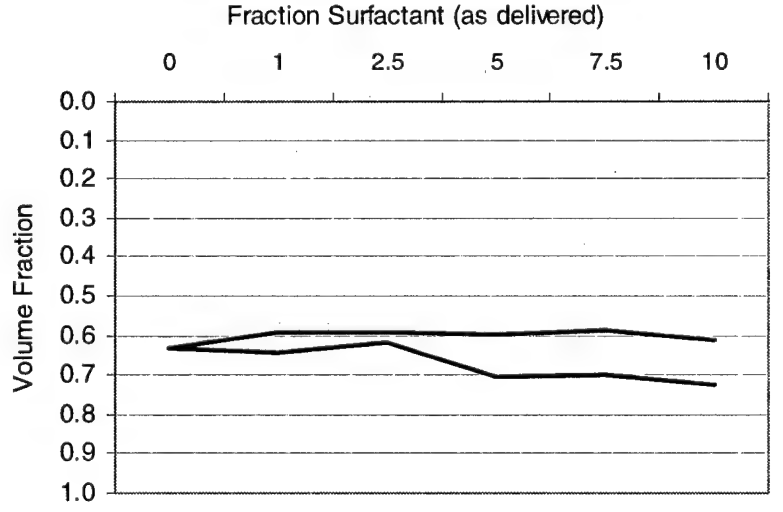
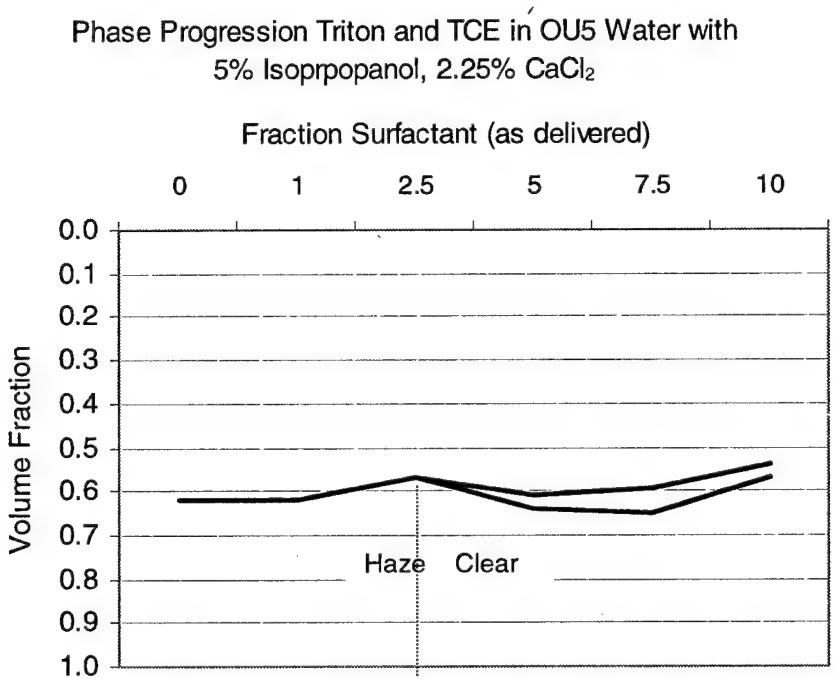


Figure 3.18:



3.3 Surfactant Screening Using Sodium Chloride and Isopropanol as Co-solvents

Screening tests continued to identify suitable ranges of surfactant in Lansing tap water. In these tests, isopropanol was used as a co-solvent in the ratio of 1:1 and 2:1 mass units of surfactant to isopropanol. Sodium chloride was used as surfactant desensitizer. Aerosol, SLS, and Rhema were used as the test surfactants. All concentrations of surfactant were on an "as delivered" basis.

One of the goals of the initial screening was to determine if stable middle-phase (micro-emulsion) or cloudy emulsion could be established as an indication of superior TCE dissolution into the water phase. The following graphs show the heights of emulsion phases (mm) established above the TCE layer, Figures 3.19 – 3.24. In all tests, 10 g TCE were contacted with 10 g of various mixtures of surfactant/water/isopropanol/salt solutions. The full heights of the materials in each test vial total about 35 mm. Tests were first performed at room temperature, and then the vials were re-shaken for tests at 10°C.

Aerosol established an iridescent blue-pearl haze within the water phase at room temperature at all surfactant concentrations from 3 through 6% at a

constant salt dose of 1% NaCl. At 10°C, the blue iridescent haze disappeared from the water phase and became established within the TCE layer.

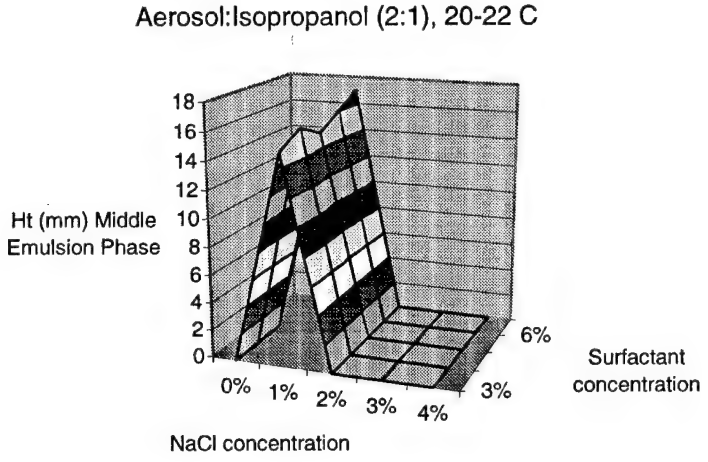


Figure 3.19: Height of Emulsion Phase Established above TCE Layer at 20-22 °C

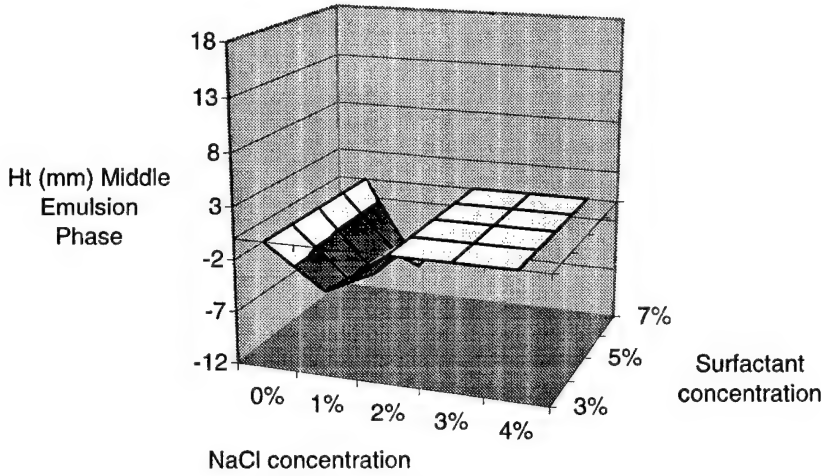


Figure 3.20: Height of Emulsion Phase Established above TCE Layer at 10 °C

Rhema produced a rich cloudy-white emulsion within the water layer that disappeared at room temperature over the course of 2-3 days. Figure 3.21 indicates that only a small layer remained. However, at 10°C, the cloudy-white emulsion remained stable for well over one week, Figure 3.22. The emulsion seemed more robust at higher salt and higher surfactant concentrations. Certainly, the cloudy-white emulsion should not be considered to be a true middle phase or microemulsion.

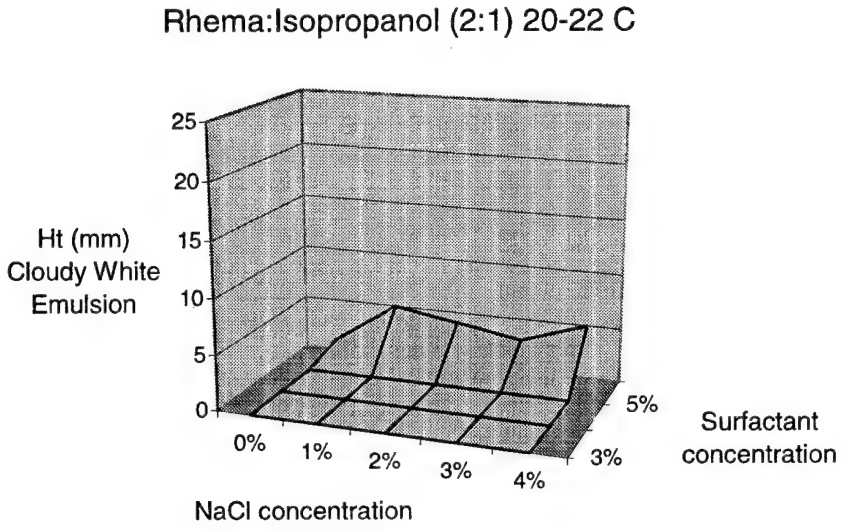


Figure 3.21: Height of Emulsion Phase Established above TCE Layer at 20-22 °C

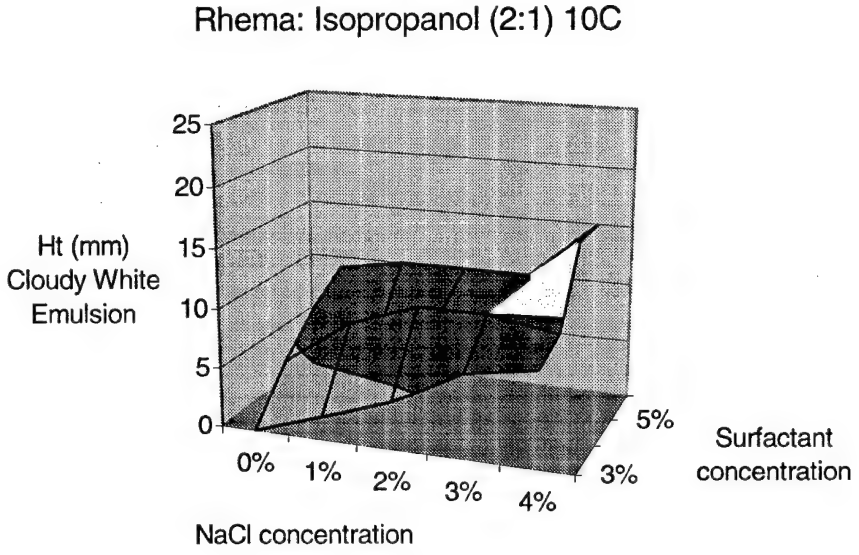


Figure 3.22: Height of Emulsion Phase Established above TCE Layer at 10 °C

SLS had a similar response to temperature, as did aerosol. At room temperature, SLS produced a clear microemulsion that could be seen as a break in the refractive index between the water phase and the microemulsion at lower salt concentrations. As the salt increased, the microemulsion became light blue to hazy, and more distinct, Figure 3.23. As the temperature was decreased to 10°C, the emulsion began to disappear, becoming non-existent at lower salt and surfactant concentrations.

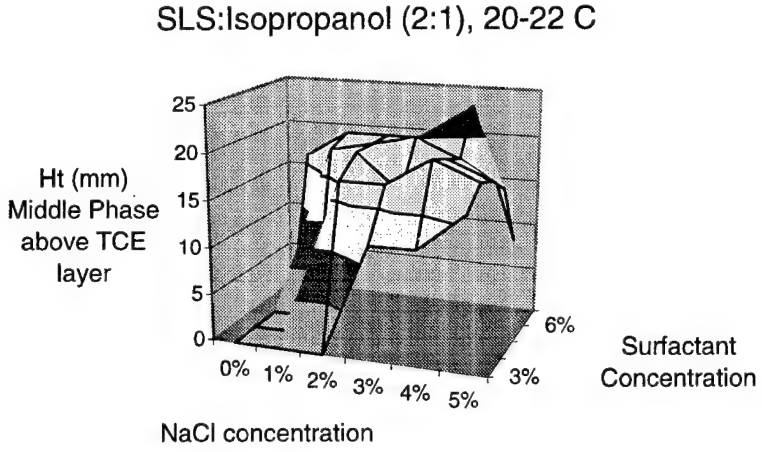


Figure 3.23: Height of Emulsion Phase Established above TCE Layer at 20-22 °C

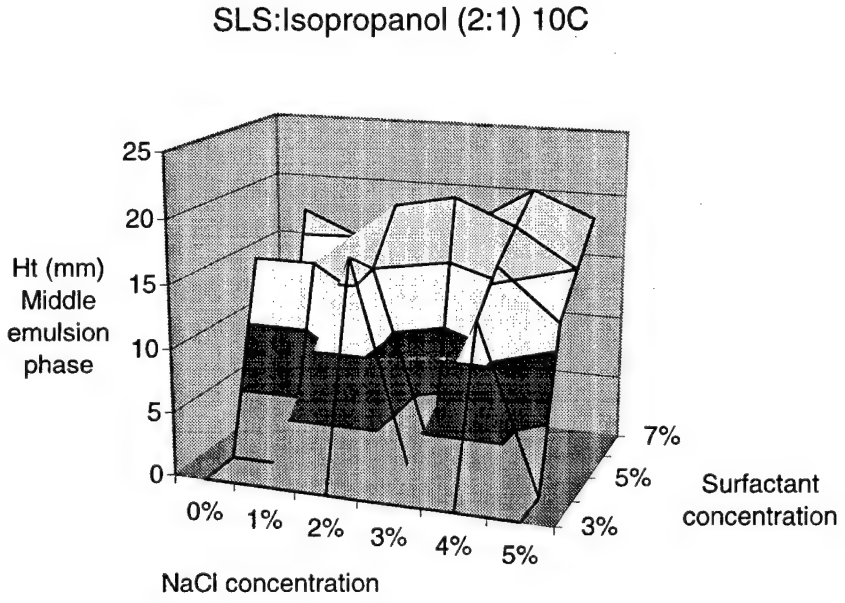


Figure 3.24: Height of Emulsion Phase Established above TCE Layer at 10 °C

3.4 Optimization: Analysis of the Emulsion, Microemulsion Phases

Another goal was to determine the best aqueous surfactant/isopropanol/salt systems that offer maximum extraction capacity of TCE from water. This was done by either contacting 2 g of TCE with 20 g surfactant mixture or 10 g TCE with 10 g surfactant mixture. In all cases, the amount of TCE used was large enough to give saturated surfactant systems upon thorough mixing. The

TCE-saturated surfactant mixtures were allowed to equilibrate for at least 48 hours before analysis.

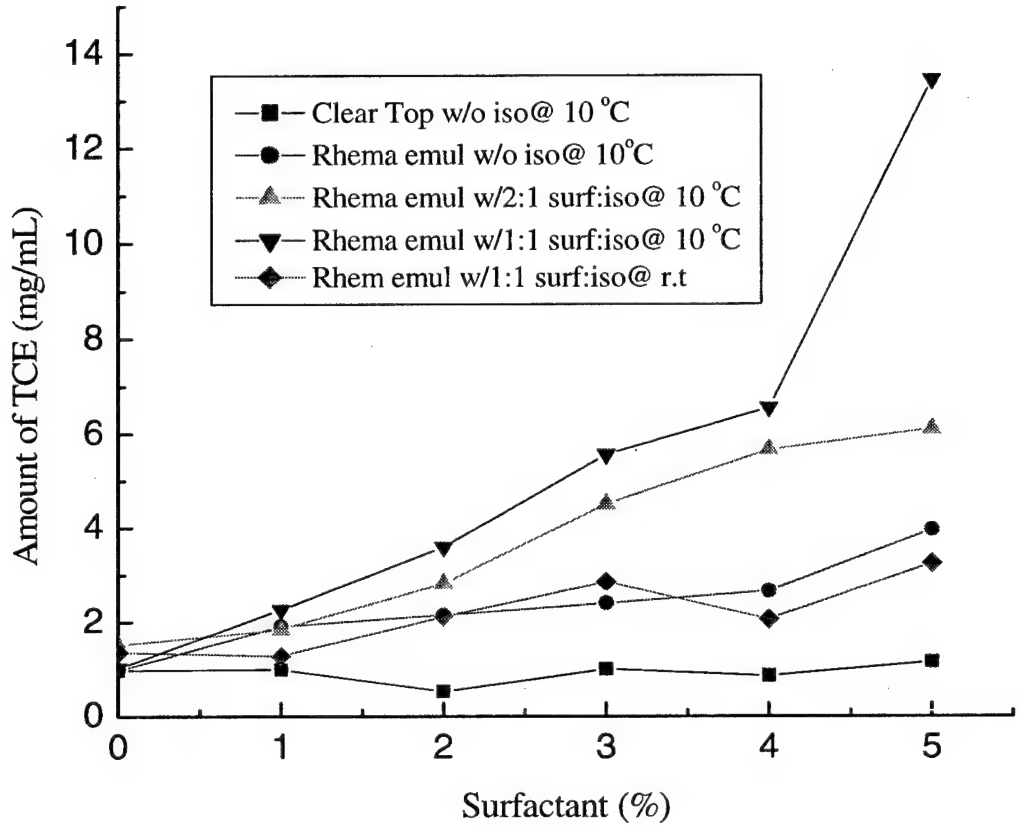
Analysis of all TCE samples (standards and unknowns) was done using HSGC. HSGC is a technique for the concentration and analysis of volatile organic compounds that makes use of the equilibrium between the volatile components of a liquid or solid sample. A headspace sample is prepared in a sealed vial containing the sample, a dilution solvent, a matrix modifier, and the surrounding gas phase or headspace. In this method, 2.5% Rhema is the dilution solvent, and sodium chloride is used as the matrix modifier.

Three surfactants, Rhema, SLS, and aerosol were evaluated for the optimum amount of surfactant, co-solvent (ispropanol), and surfactant desensitizer (sodium chloride). Various TCE-saturated surfactant/isopropanol/salt (0.25 mL) were analyzed by HSGC and the TCE and PCE peak areas recorded. Calibration standards are analyzed together with all unknown samples and fresh calibration plots generated for each batch of samples. In all cases, the amount of sample injected was consistent and thus peak areas were used for the calibration plots and for calculating amounts of unknown TCE.

Rhema/Isopropanol/ Salt systems: Aqueous solutions (20 mL) containing 0, 1, 2, 3, 4, 5% Rhema were contacted with 2 g TCE and allowed to equilibrate for 48 hours at 10° C. Various amounts of a whitish emulsion were formed in each case except in the solution with 0% Rhema. The height of emulsion formed in each case was noted and 0.25 mL of emulsion analyzed for TCE content. The clear, non-emulsion top layers were also analyzed for TCE content. Isopropanol was then added to each solution to form a 2:1 Rhema:isopropanol mixtures, shaken to mix thoroughly and allowed to equilibrated for another 48 hours at 10° C and the height of emulsion and TCE content determined. More isopropanol was added to the solution mixtures to obtain 1:1 Rhema:isopropanol and again, allowed to equilibrate at 10° C , and the height of emulsion and TCE content determined. Figure 3.25 shows variations of the amount of TCE (mg/mL) for the various Rhema concentrations and the two temperatures.

From Figure 3.25, it can be concluded that for the surfactant concentrations studied, the optimum Rhema concentration is 5 % as it carries the most TCE. In addition, a 1:1 Rhema: Isopropanol carries more TCE than 2:1 TCE:isopropanol and more TCE is carried at 10° C than at room temperature.

Figure 3.25: Variation of TCE Content with Surfactant Concentration.



Another set of samples containing 3 – 5% of Rhema (10 g each) were contacted with 10 g TCE, allowed to equilibrate and the TCE content analyzed. It was observed that 4 and 5% Rhema carried much more TCE than the rest of the other Rhema concentrations.

Sodium chloride, used as a surfactant desensitizer, was optimized for 4 and 5% Rhema solutions containing 1:1 surfactant:isopropanol at 10ºC and at room temperature. This was done by adding various amounts of sodium chloride to 4 and 5% Rhema solutions to give 0, 1, 2, 3, 4% salt solutions with respect to the surfactant content. These solutions (10 g) were contacted with 10 g TCE allowed to equilibrate for 48 hours at 10° C and room temperature and the TCE content in the aqueous phase determined by GC. A plot of the amount of TCE versus percent salt, Figure 3.26, showed an optimum salt concentration of 3% for both 4 and 5% Rhema at 10° C. At room temperature however, salt did not appear to have any effect on the surfactant's ability to carry TCE.

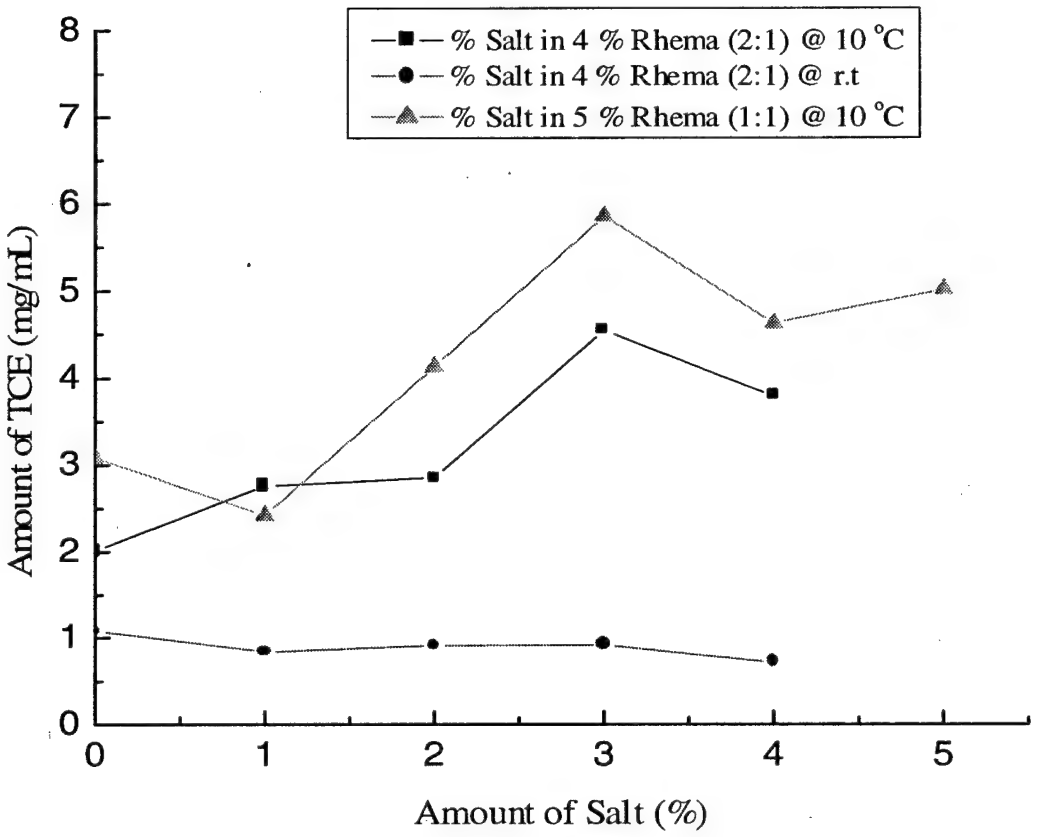


Figure 3.26: Plot of Variation of TCE content with Salt Concentration in Rhema Mixture

SLS/Isopropanol/Salt Systems: Aqueous solutions containing 3, 4, 5, 6, and 7% Sodium Lauryl Sulfate (SLS) were prepared and isopropanol added to obtain SLS:isopropanol concentrations of 2:1. Sodium chloride was then added to separate surfactant mixtures to give salt concentrations of 1-5% salt with respect to SLS. Each surfactant/isopropanol/salt mixture (10 g) was contacted by 10 g TCE, allowed to equilibrate at 10° C and at room temperature (~ 22° C) for about 48 hours. Very little emulsion formed in solutions containing 1 and 2% salt, whereas, visibly large amounts of microemulsions were formed in solutions containing 3 – 5% salt. These emulsions were analyzed for their TCE content using headspace gas chromatography. Figure 3.27 shows the variation of TCE content with amount of surfactant and salt used. Clearly, for a 2:1 SLS:isopropanol mixture, 5% surfactant and 3 to 4% salt appear to be the optimum mixture for room temperature extractions. SLS tends to do very poorly at 10° C as shown in Figure 3.27.

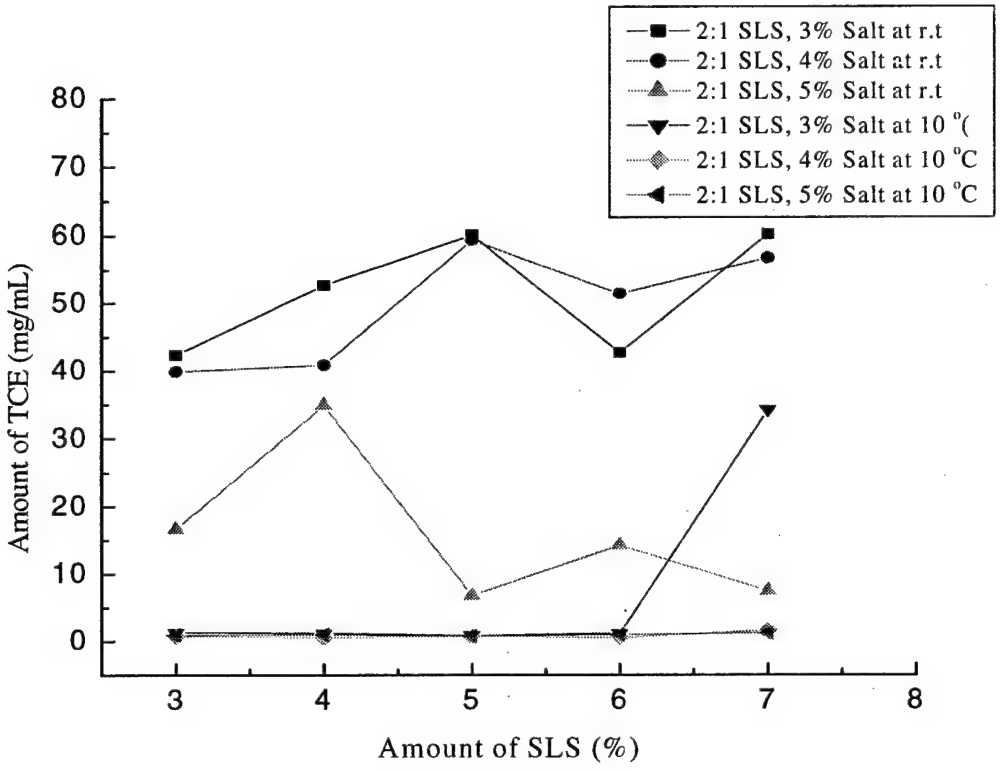


Figure 3.27: Variation of Amount of TCE with SLS and Salt Concentrations

Aerosol/Isopropanol/Salt Systems: Isopropanol was added to 1 – 10% aerosol solutions to obtain aerosol:isopropanol concentrations of 2:1 and then sodium chloride was added to give 0-5% salt with respect to aerosol concentration. Each aerosol mixture (10 g) was added to 10 g TCE and allowed to equilibrate at room temperature for about 48 hours. Microemulsions were formed in solutions containing 0-2% salt and these were analyzed using GC for the TCE content. Another aerosol solution containing 2:1 aerosol:isopropanol and 1% salt was contacted with TCE and allowed to equilibrate at 10° C. Figure 3.28 shows the variation of TCE in the aerosol microemulsion with surfactant and salt concentrations.

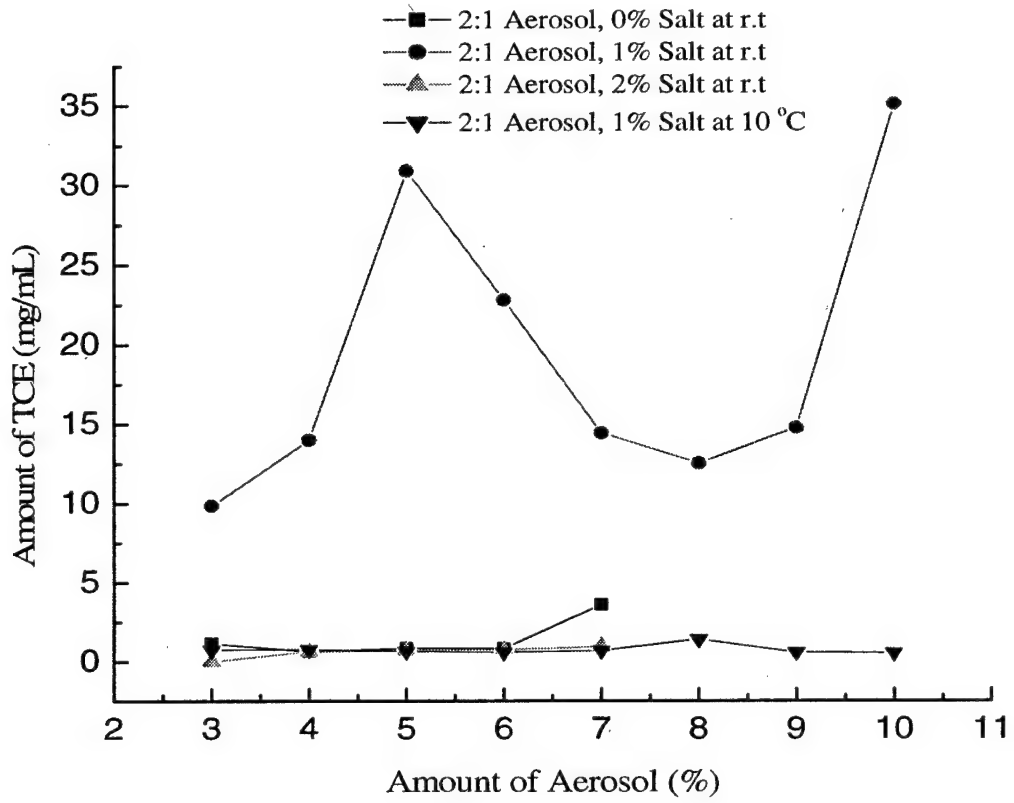


Figure 3.28: Variation of Amount of TCE with Temperature as well as Surfactant and Salt Concentrations

From Figure 3.28 we can conclude that:

1. Aerosol:isopropanol (2:1) carries more TCE at room temperature than at 10°C
2. Aerosol with 1% salt carries more salt than 2% or no salt.
3. Optimum aerosol that gives maximum capacity for TCE is 5%

Temperature Effects on TCE Content in 1:1 and 2:1 Rhema, SLS, and Aerosol Solutions: Temperature effects on the concentration of TCE in various surfactant mixtures were investigated and an Arrhenius plot generated. This was done by contacting TCE with 1:1 and 2:1 surfactant (Rhema, SLS and aerosol)/isopropanol solutions containing the optimized salt concentrations of 3% for Rhema and SLS and 1% for aerosol. One sample of each mixture was allowed to equilibrate at 4, 10, 14, and 22° C for a 48 hours and the TCE content analyzed by GC. The Arrhenius plot shown in Figure 3.29 show that SLS carries a lot of TCE at room temperature but very little at 10° C. Aerosol carries relatively the same amount of TCE at all the

temperatures comparable to the amount carried by SLS at room temperature. On the other hand, Rhema mixtures carry relatively large amounts of TCE at all temperatures but Rhema mixtures tend to form cloudy white emulsions while SLS and aerosol form clear blue microemulsions. However, 2:1 Rhema:isopropanol solutions appear to carry slightly more TCE at lower temperatures than 1:1 mixtures.

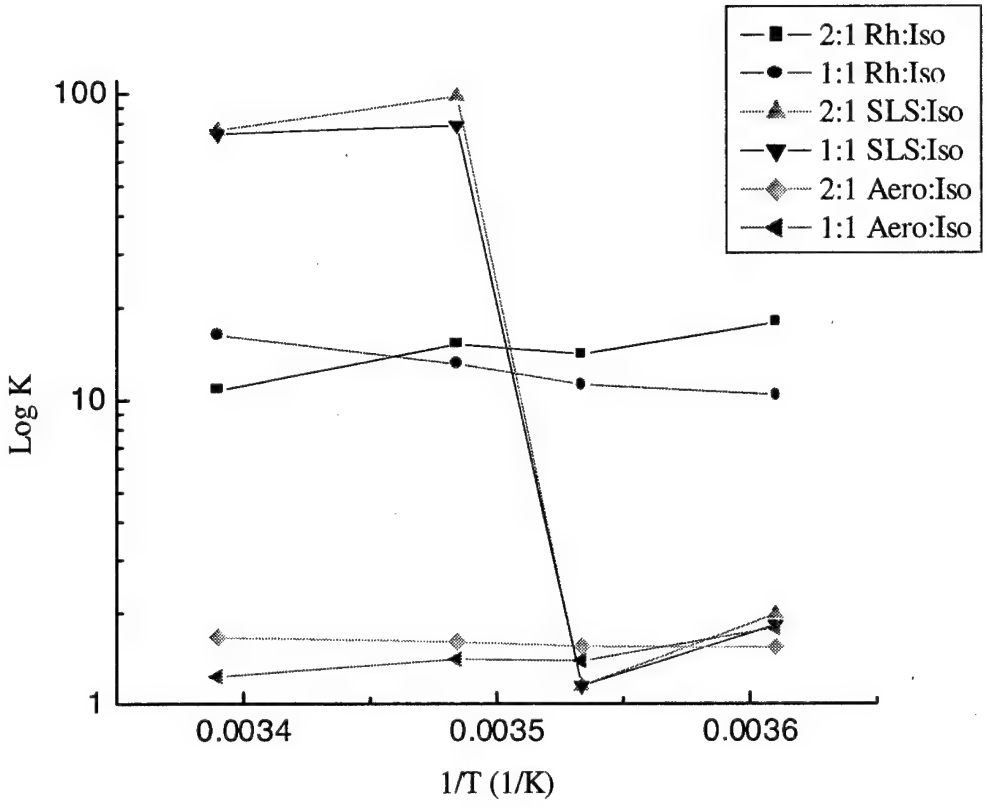


Figure 3.29: Temperature Dependence of TCE Concentration in Surfactant Mixtures

Rhema/SLS Mixtures: Since the clear blue microemulsion of SLS carries a lot of TCE at room temperature and the emulsion of Rhema is good at both temperatures, mixtures of the two surfactants in different proportions were investigated and compared to pure Rhema/isopropanol and pure SLS/Isopropanol solutions. The mixtures investigated were 1:1 Rhema:SLS and 4:1 Rhema:SLS, each containing 3% salt and 1% isopropanol. In addition, 1:1 Rhema:isopropanol and 1:1 SLS:isopropanol, each containing 3% salt were investigated. All four sets of sample mixtures were contacted with TCE and allowed to equilibrate at 4, 10, 14, and 22° C for 48 hours. The amount of TCE was determined by GC and an Arrhenius plot generated, Figure 3.30.

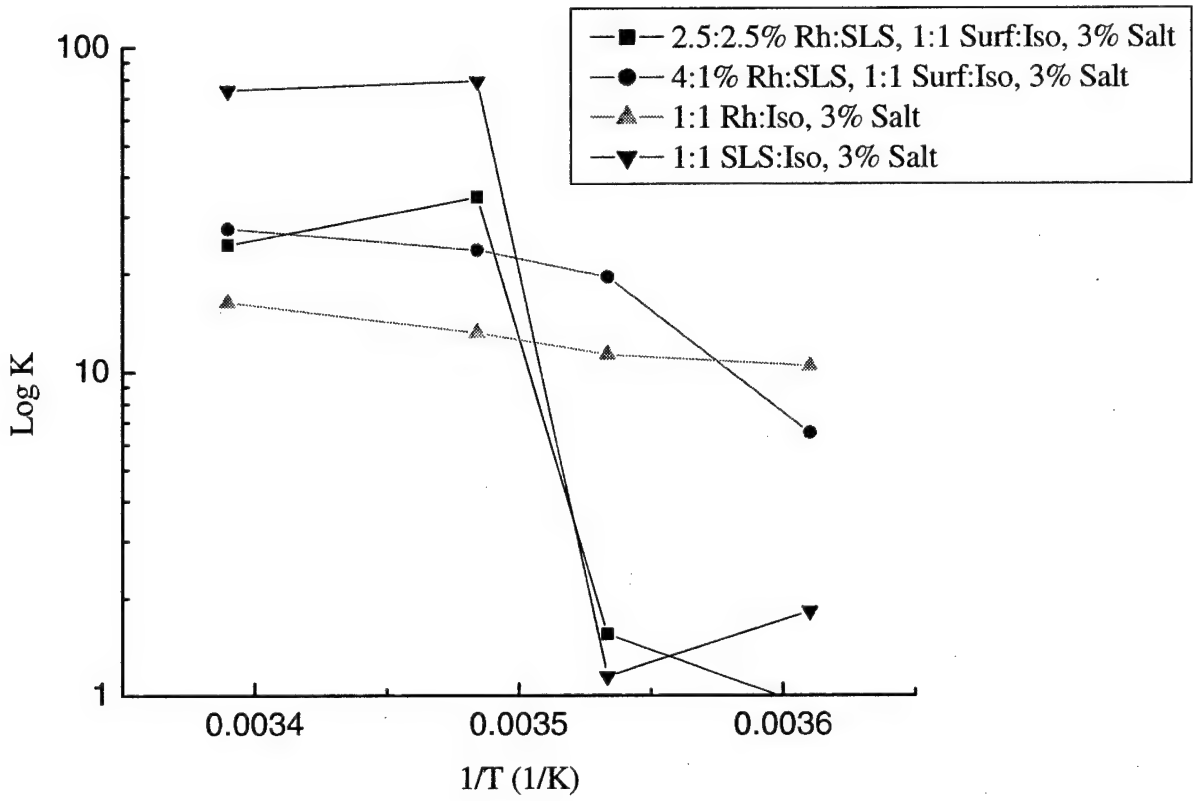


Figure 3.30: Temperature Dependence of TCE Concentration on Surfactant Mixtures

The results indicated that equal amounts of SLS and Rhema tend to follow the same trend as SLS/isopropanol over the temperature range studied, carrying more TCE at room temperature than at lower temperatures. A 4:1 Rhema:SLS mixture on the other hand, behaves similarly to Rhema/isopropanol but carries a little more TCE at all temperatures except at 4° C. The amount of TCE carried by this system however, is still considerably less than that carried by SLS/isopropanol at room temperature and at temperatures lower than 10° C, the amount of TCE decreases sharply. The temperature dependence of TCE concentration in 5:1 and 6:1 Rhema/SLS mixtures containing 3% salt and 1:1 surfactant:isopropanol was also investigated. The results are shown in Figure 4.31 together with those from Figure 3.30 for comparison.

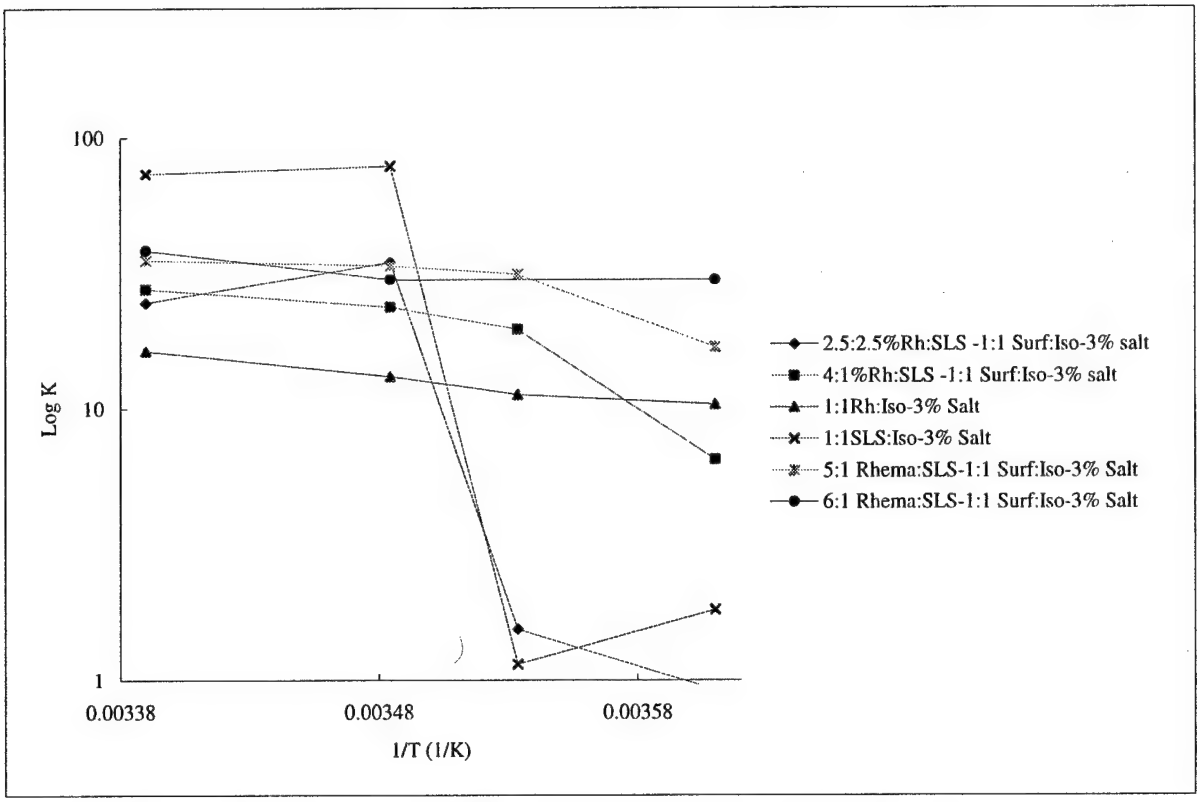


Figure 3.31: Temperature Dependence of TCE Concentration in 5:1 and 6:1 Surfactant Mixtures

From Figure 3.31, it is obvious that higher Rhema to SLS ratios (5:1 and 6:1) carry one TCE at all temperatures than lower ratio mixtures. Most notable is the fact that 6:1 Rhema:SLS carries more TCE at temperatures lower than 10° C than all the other mixtures and this effect appears to increase with decreasing temperature. It is entirely possible that higher than 6:1 Rhema to SLS ratios will carrier even more.

Figure 3.32 summaries the TCE carrying capacity of optimized Rhema, SLS, and aerosol as well as Rhema/SLS mixtures at 10° C. Clearly, the Rhema/SLS mixtures carry more TCE than the pure surfactants with the optimum being 5:1 Rhema:SLS. In addition, these mixtures form stable, clear blue microemulsions similar to those of pure aerosol and SLS. Since Utah ground water has a temperature of about 10° C, it may be more helpful to use a combination of Rhema and SLS for soil TCE remediation than the currently used surfactant mixture (2:1 aerosol, 1% salt). Surfactant mixtures in Utah waters will be optimized bearing in mind parameters used in the Lansing tap water investigations.

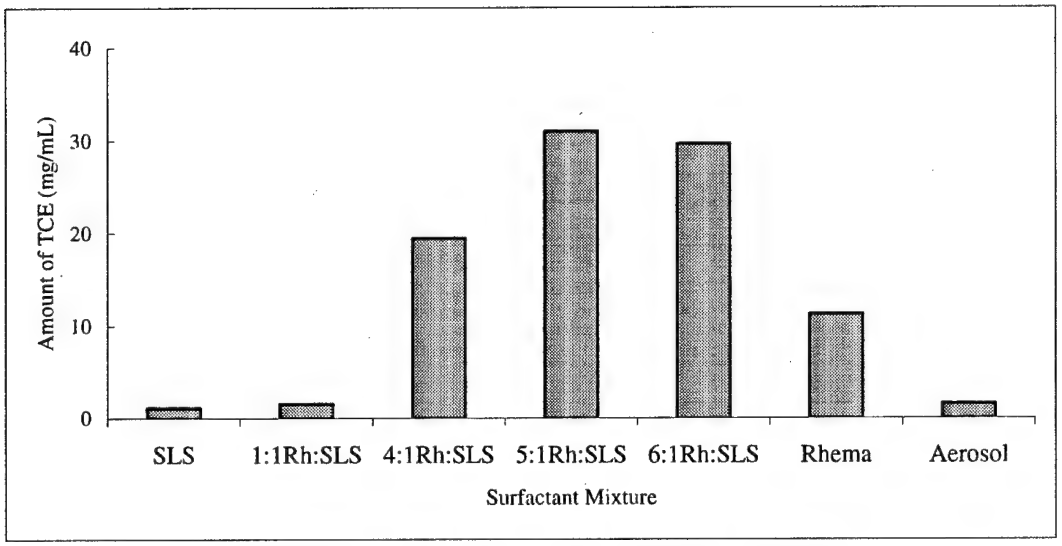


Figure 3.32: Summary of TCE Carrying Capacity of Various Surfactant Mixtures at 10° C

The optimum surfactant/isopropanol/salt mixtures for Rhema and SLS been established in Lansing tap water to be 5% surfactant, 1:1 surfactant:isopropanol, and 3% salt, while that for aerosol is 5% surfactant, 2:1 surfactant:Isopropanol, and 1% salt. With these optimums, we have shown that:

- Rhema carries more TCE than aerosol at all temperatures studied, though Rhema forms an emulsion and SLS forms a clear blue microemulsion.
- Rhema carries more TCE at 10° C than SLS whereas at room temperature, SLS carries much more TCE and Rhema and aerosol.
- SLS carries very little TCE at 10° C comparable to that of aerosol but carries very large amounts of TCE at higher temperatures.
- Mixtures of Rhema and SLS tend to increase the amount of TCE carried in the aqueous surfactant solution.
- Higher ratios of Rhema to SLS tend improve the TCE carrying capacities of the aqueous mixtures.

Optimization in Utah Site Waters OU1, OU2, OU5, and OU12:

The evaluation of the surfactants; Rhema, aerosol, and blended Rhema:SLS, in the Utah waters was performed by selecting a range of salt and surfactant concentrations based on the optimum conditions established with Lansing tap water at 10° C. The matrices tested in the Utah water at 10° C were:

- 1:1 Rhema:isopropanol with 3, 4, and 5% surfactant and a range of 2, 3, and 4% sodium chloride.

- 2:1 Aerosol:isopropanol with 3, 4, and 5% surfactant and a range of 0.5, 1, and 1.5% sodium chloride.
- 1:1 Rhema/SLS:ispropanol with the surfactant blend based on 6:1 Rhema:SLS and having a total surfactant concentrations of 4, 5, and 6% and a range of 2, 3, and 4% sodium chloride.

The amount of TCE in the surfactant mixtures was determined by headspace GC using PCE as internal standard. Phase diagrams showing the amount of TCE (mg/mL), salt (%), and surfactant (%) were plotted and optimum surfactant mixtures determined and used for column extractions of TCE contaminated Utah soils.

Utah Water Samples:
Rhema, aerosol, and Rhema/SLS blend solutions (20 mL each) containing different amounts (%) of surfactant, isopropanol, and salt were contacted with 2 g TCE and allowed to equilibrate for 48 hours at 10° C. Various amounts of emulsions and microemulsions were formed and their characteristics and behavior under different conditions are described below.

Characteristics of Emulsions and Microemulsions formed:
The Utah ground water temperature is determined to be 10° C, therefore, TCE/surfactant mixtures were allowed to equilibrate at this temperature for 48 hours. These formed emulsions and microemulsions that vary with surfactant composition and temperature as described below.

Figure 4.33 is an example of the micro-emulsion that can be generated with aerosol, salt, and isopropanol. In this figure, the various site waters, OU1, OU2, OU5, and OU12 were treated with 4% aerosol, 0.5% sodium chloride and a constant ratio of 2:1 surfactant to isopropanol by weight and contacted with 2 g TCE per 20 g of test solution. The samples were refrigerated for 21 days prior to taking the photo, indicating that the blue haze represents a true micro-emulsion. The solution is actually clear to hazy white with the pearl like blue color being created by refraction of light. Note that the OU1 water is nearly tri-phasic with a heavy white haze above the micro-emulsion. All samples show the remaining TCE layer as a heavy white emulsion in the bottom of each tube.

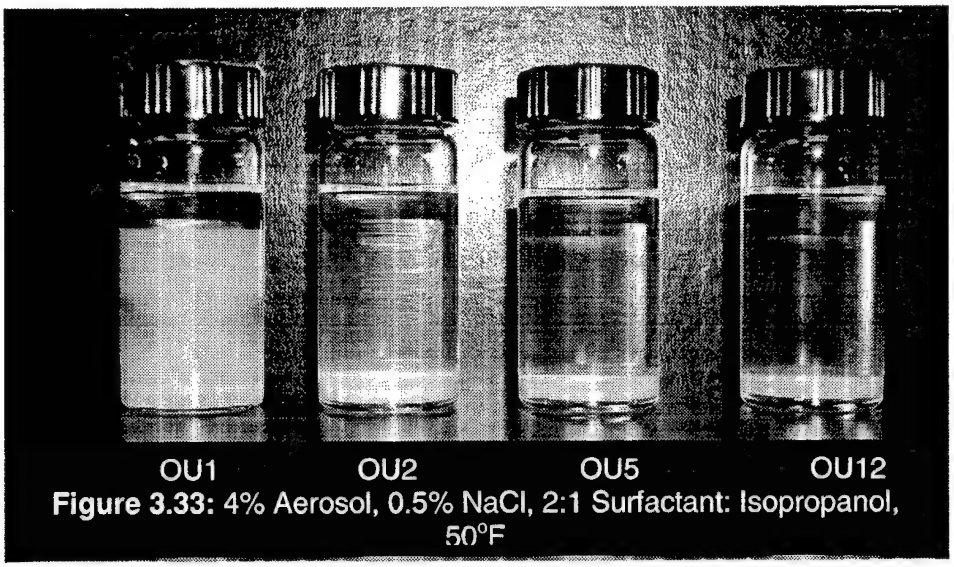


Figure 3.33: 4% Aerosol, 0.5% NaCl, 2:1 Surfactant: Isopropanol, 50°F

The aerosol system is highly sensitive to temperature, salt, and co-solvent concentrations. The tubes in Figure 3.34 were prepared as stated for Figure 3.33 with the exception that 1% NaCl was used instead of 0.5% NaCl. The preparation was 4% aerosol, 1% sodium chloride and a constant ratio of 2:1 surfactant to isopropanol by weight and contacted with 2 g TCE per 20 g of test solution. Notice that the top water layers are clear and that the blue haze micro-emulsion has disappeared into the heavy TCE layer.

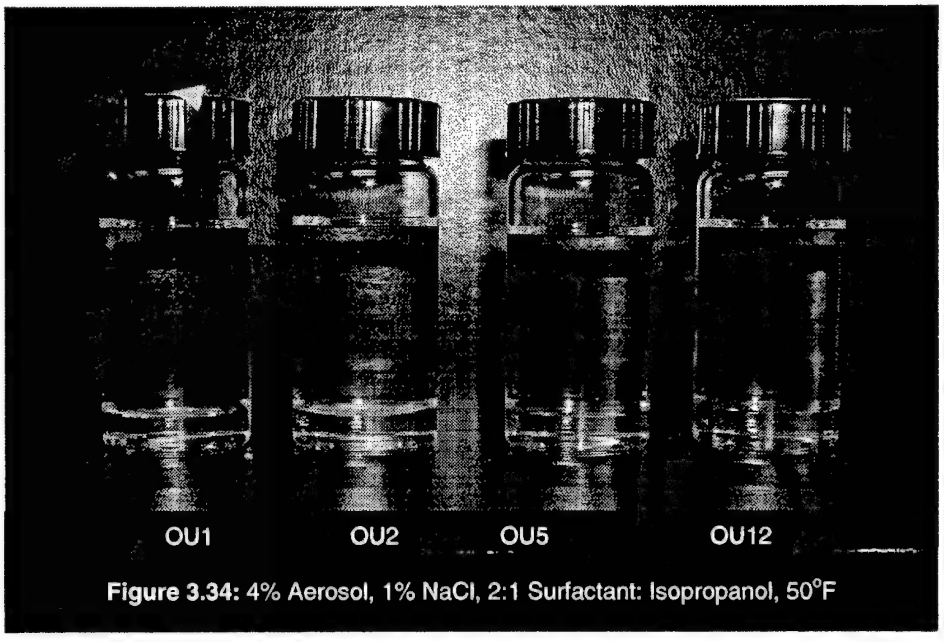


Figure 3.34: 4% Aerosol, 1% NaCl, 2:1 Surfactant: Isopropanol, 50°F

Figure 3.35 is a typical example for Rhema contacted with TCE. This particular preparation represents 5% Rhema, 4% sodium chloride, and a 1:1 ratio of surfactant to isopropanol. The white phase is a true emulsion that is unstable after about 2 weeks at 50° F. Passing the white haze through a

syringe needle will break the emulsion. The presence of the true emulsion makes Rhema a poor candidate for surfactant flooding.

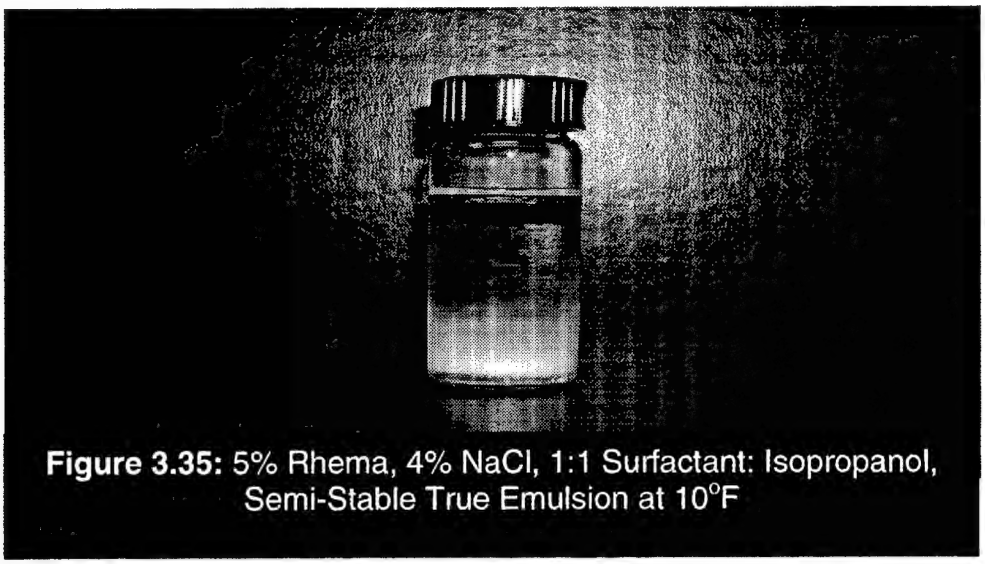


Figure 3.35: 5% Rhema, 4% NaCl, 1:1 Surfactant: Isopropanol, Semi-Stable True Emulsion at 10°F

SLS produce a micro-emulsion in TCE. Figure 3.36 shows a preparation of 20 g 3% SLS, 1% NaCl and 1:1 surfactant to isopropanol plus 2 g TCE. The blue haze is indicative of a micro-emulsion. This example is at room temperature. The SLS micro-emulsion is temperature sensitive and fails to form at temperatures less than about 65° F.

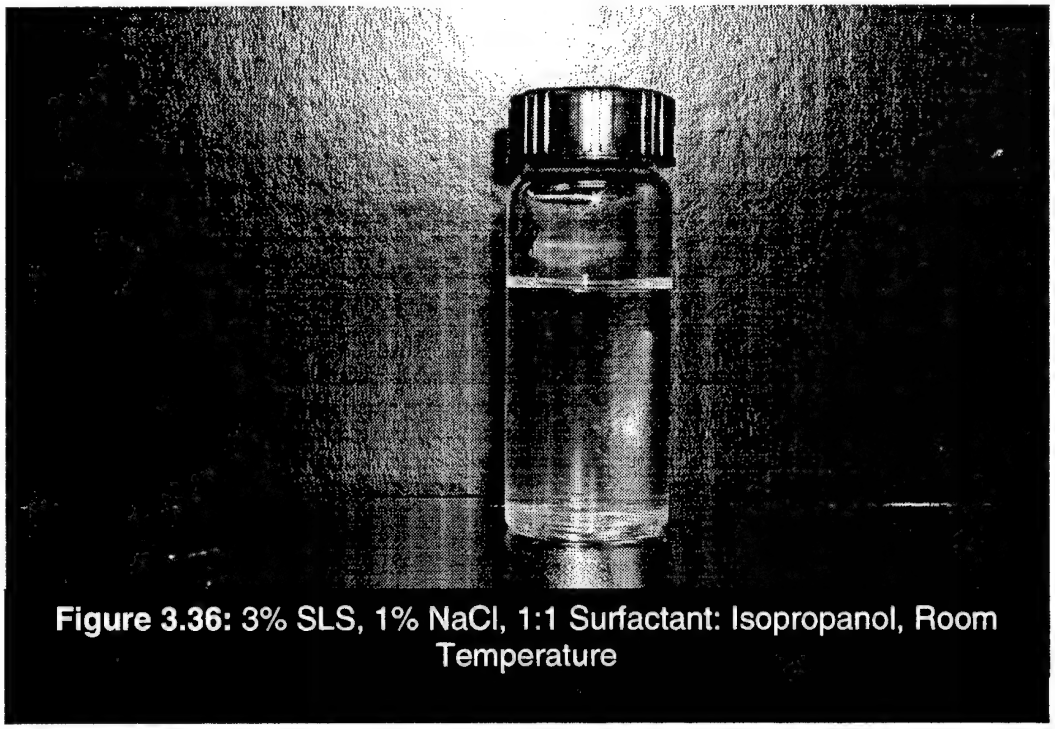


Figure 3.36: 3% SLS, 1% NaCl, 1:1 Surfactant: Isopropanol, Room Temperature

Effort was expended to determine if the combination of SLS and Rhema would produce a stable micro-emulsion that exhibits the temperature insensitivity of the Rhema true emulsion with the micro-emulsion properties of SLS. Figure 3.37 shows a preparation of 5% total surfactant containing a blend of 6:1 Rhema to SLS with a surfactant to isopropanol ratio of 1:1 stored at room temperature. It is important to note that the blue haze, indicative of the micro-emulsion is also present in this preparation at 50° F.

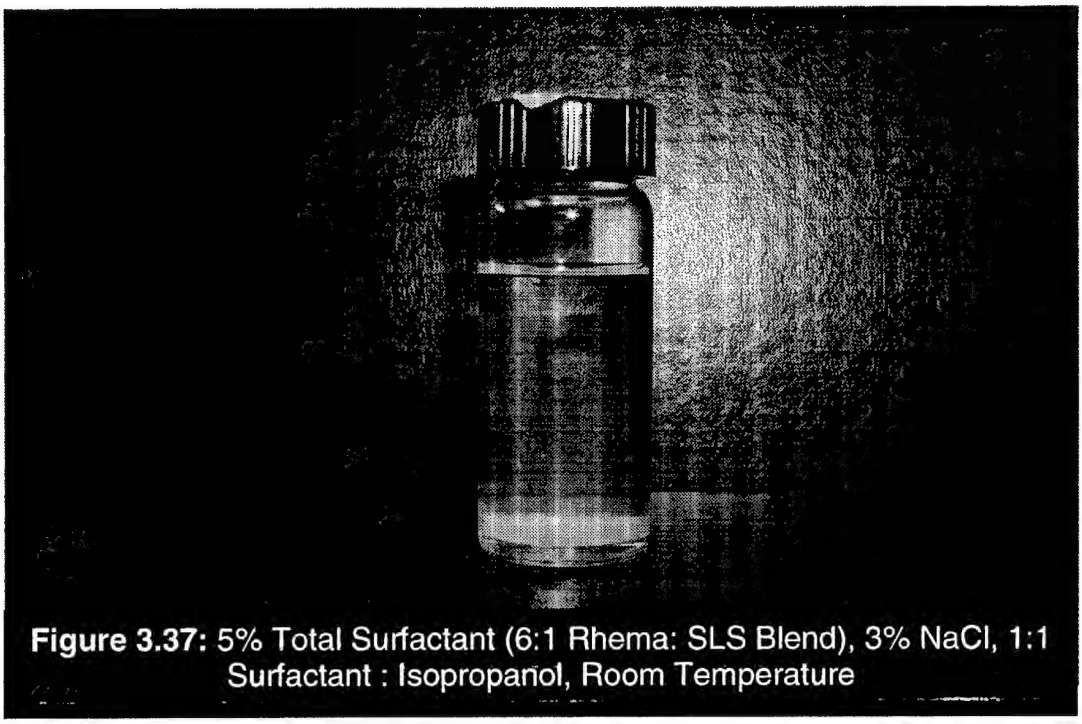


Figure 3.37: 5% Total Surfactant (6:1 Rhema: SLS Blend), 3% NaCl, 1:1 Surfactant : Isopropanol, Room Temperature

Figure 3.38 shows a series of Rhema: SLS blends prepared using the OU site samples. The preparation is 5% total surfactant containing a blend of 6:1 Rhema to SLS with a surfactant to isopropanol ratio of 1:1 stored at 50° F. This demonstrates that certain surfactants may be blended to create an active mixture that has the best properties of the individual components and minimizes the undesirable properties.

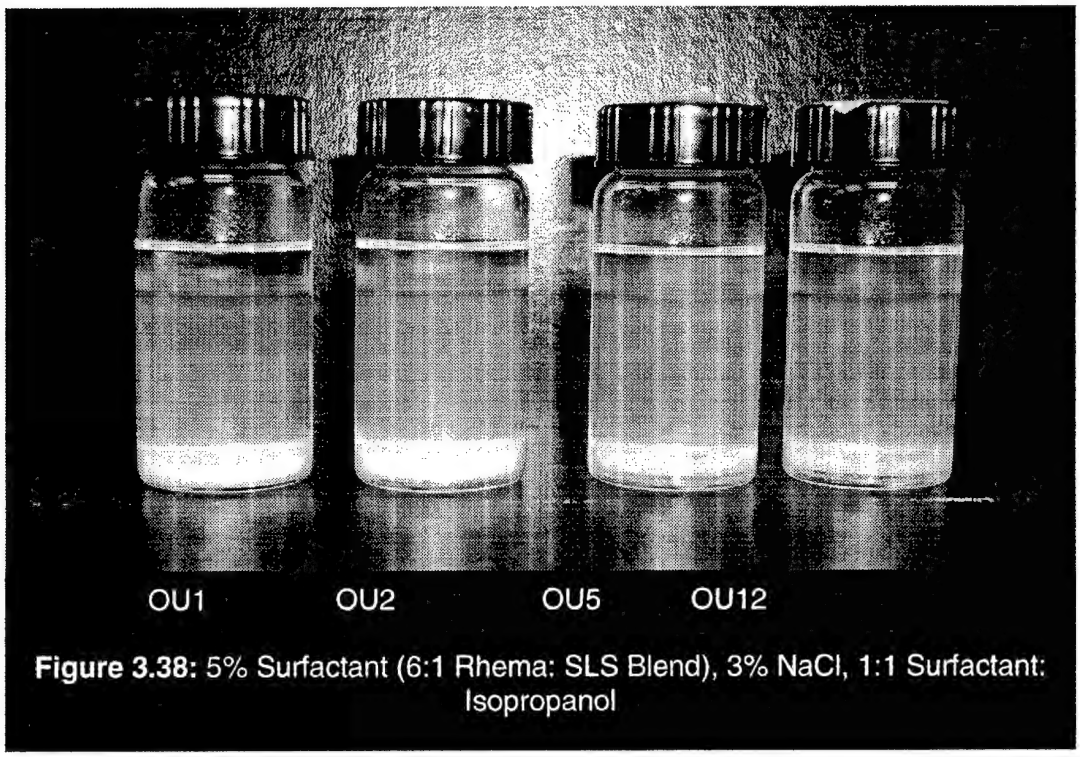


Figure 3.38: 5% Surfactant (6:1 Rhema: SLS Blend), 3% NaCl, 1:1 Surfactant: Isopropanol

Amount of TCE in Emulsions/Microemulsions:
The amount of TCE was determined for the following surfactant compositions at 10° C in four Utah waters identified as OU1, OU2, OU5, and OU12.

Rhema: 1:1 surfactant:isopropanol solutions containing 3, 4, and 5% Rhema and 2, 3, and 4% salt

Aerosol: 2:1 Aerosol:isopropanol solutions mixtures containing 3, 4, and 5% surfactant and 0.5, 1, and 1.5 % Salt

Rhema/SLS blend: 6:1 Rhema:SLS mixtures having total surfactant concentrations of 4, 5, and 6 %surfactant and 2, 3, and 4 % salt.

The TCE-saturated emulsions and microemulsions formed (0.25 mL) were analyzed by HSGC to determine the amount (mg/mL) of TCE they carried. Calibration standards were analyzed together with all unknowns and fresh plots generated for each sample batch. In all cases, the amount of sample injected was consistent, as per the peak areas of the internal standard, and thus peak areas were used for the calibration plots and for calculating the amount of TCE in the emulsions/microemulsions. Phase diagrams showing the amount of TCE in mg/mL, % salt, and % surfactant were generated from which the optimum surfactant compositions were determined. The phase diagrams for Rhema are shown in Figures 3.39 – 3.42 while aerosol and

Rhema/SLS blend are shown in Figures 3.43 – 3.46 and Figures 3.47 – 3.50 respectively.

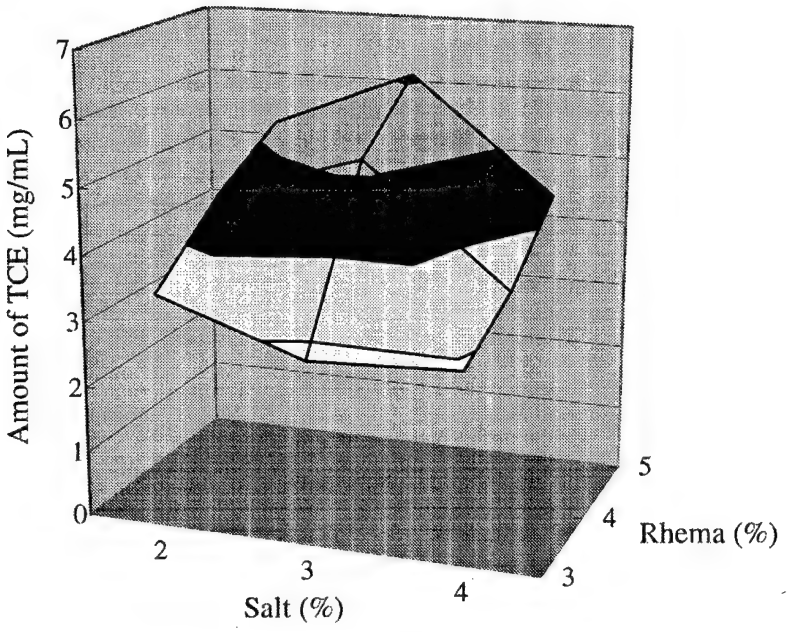


Figure 3.39: Phase Diagram for TCE in 1:1 Rhema:Isopropanol in OU1 at 10 °C

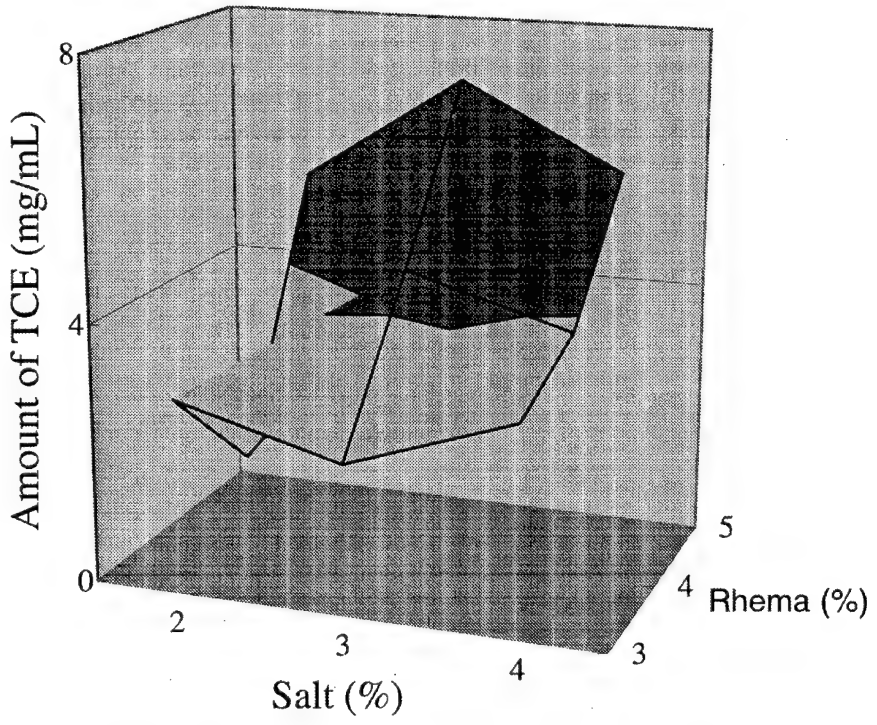


Figure 3.40: Phase Diagram for TCE in 1:1 Rhema:Isopropanol in OU2 at 10 °C

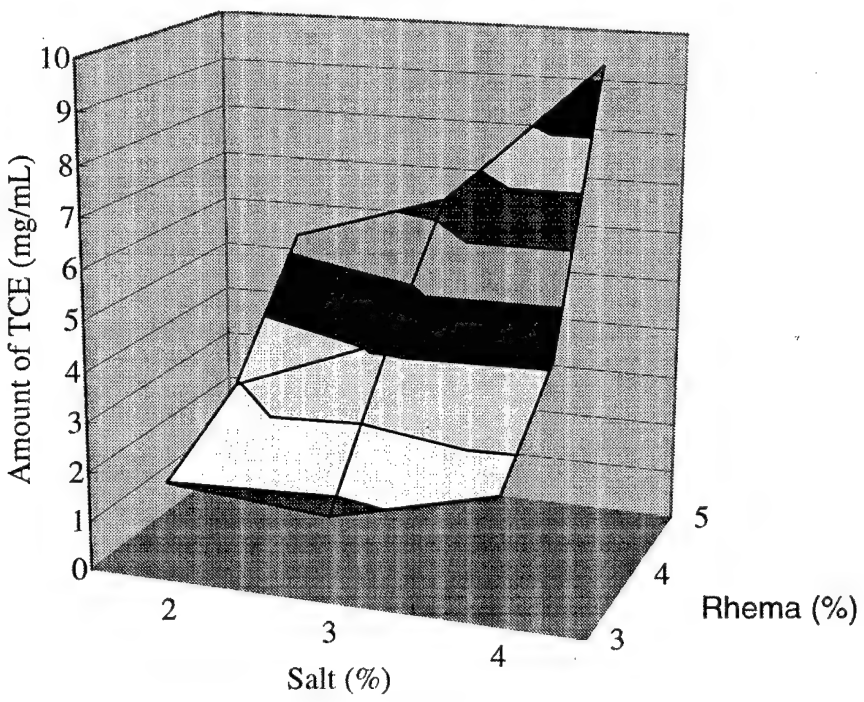


Figure 3.41: Phase Diagram for TCE in 1:1 Rhema:Isopropanol in OU5 at 10 °C

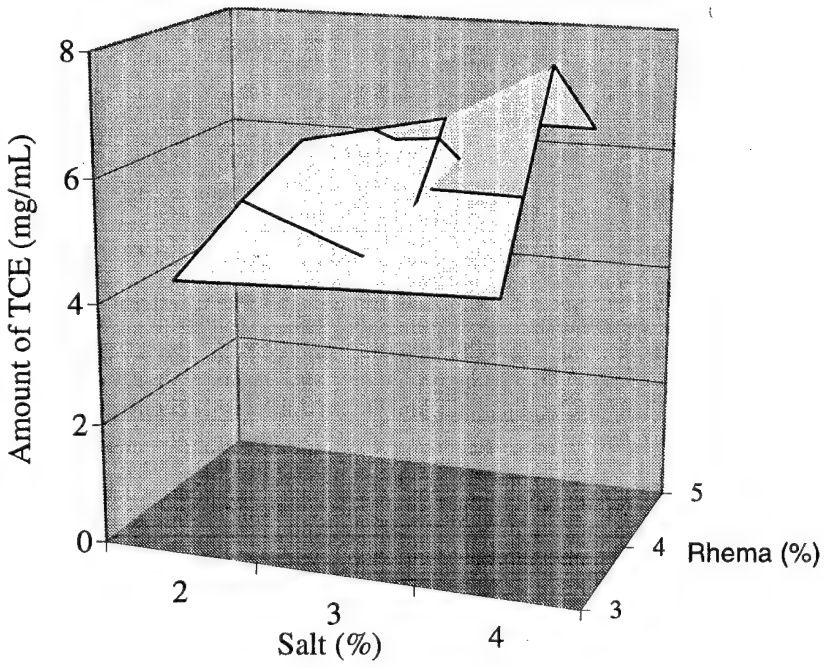


Figure 3.42: Phase Diagram for TCE in 1:1 Rhema:Isopropanol in OU12 at 10 °C

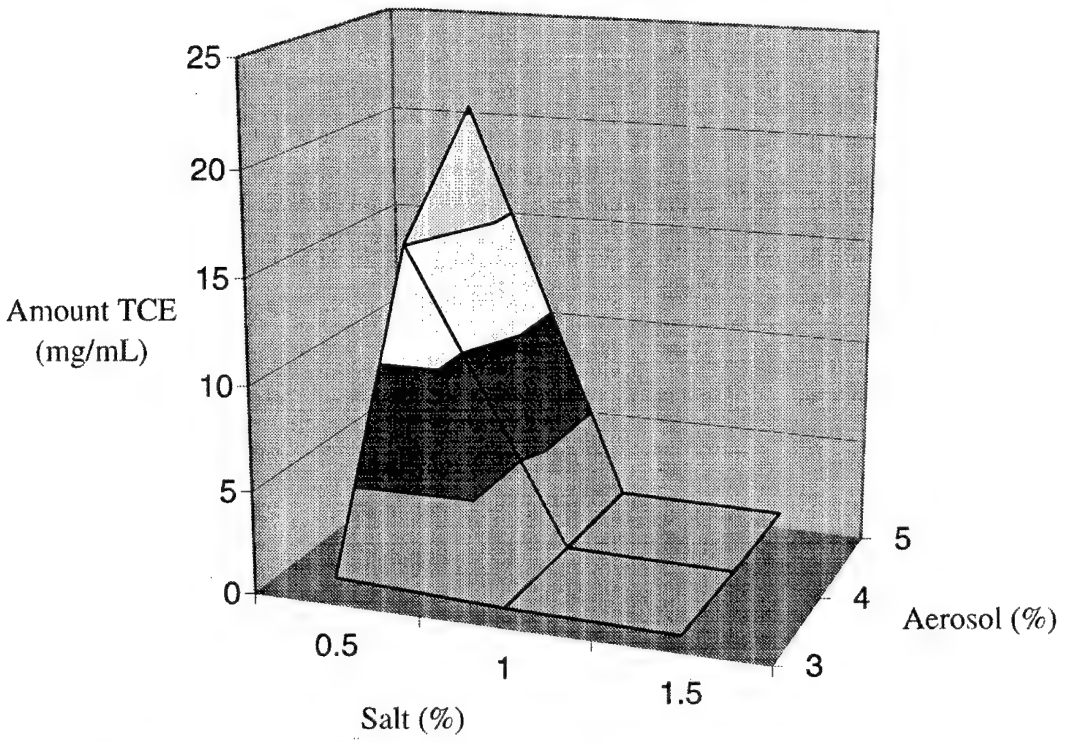


Figure 3.43: Phase Diagram for TCE in 2:1 Aerosol:Isopropanol in OU1 at 10 °C

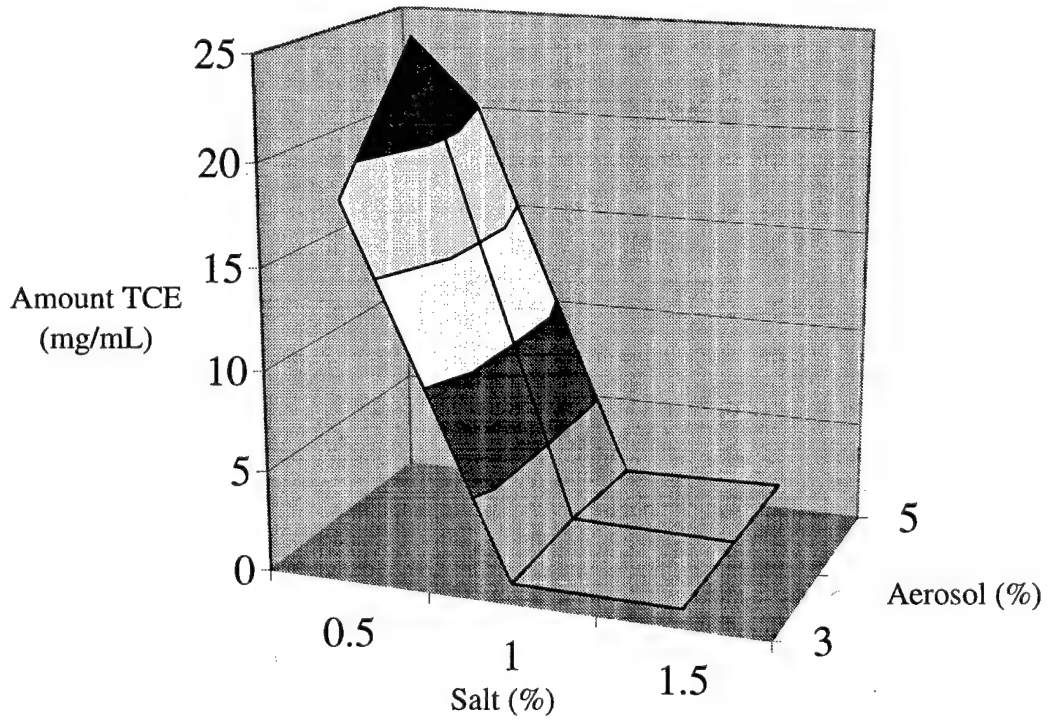


Figure 3.44: Phase Diagram for TCE in 2:1 Aerosol:Isopropanol in OU2 at 10 °C

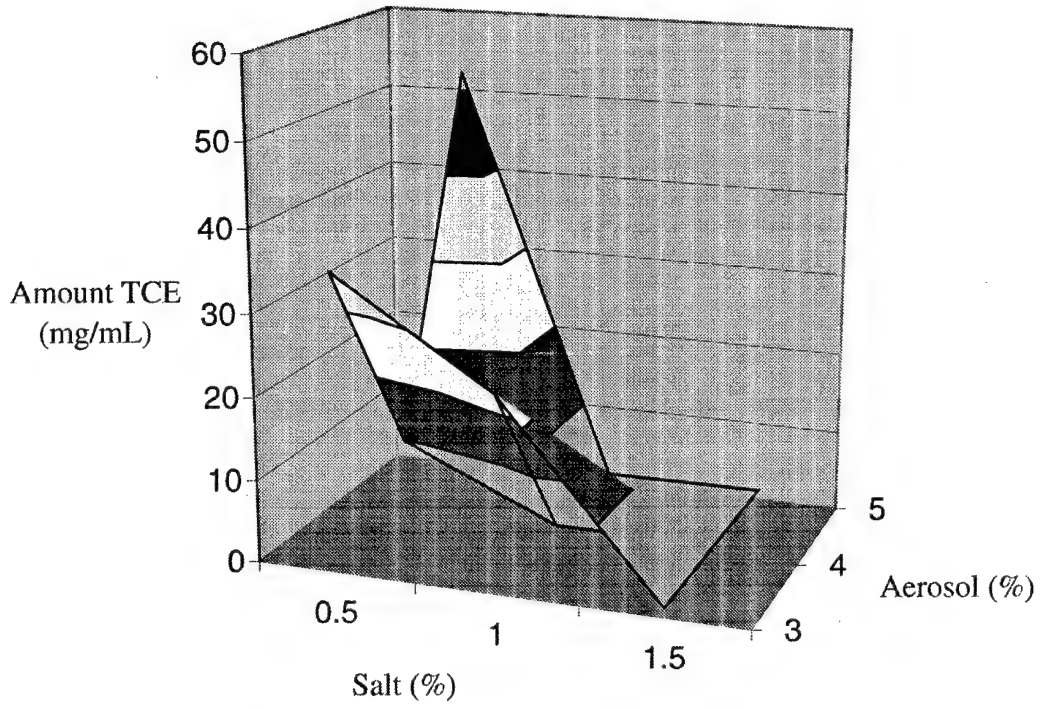


Figure 3.45: Phase Diagram for TCE in 2:1 Aerosol:Isopropanol in OU5 at 10 °C

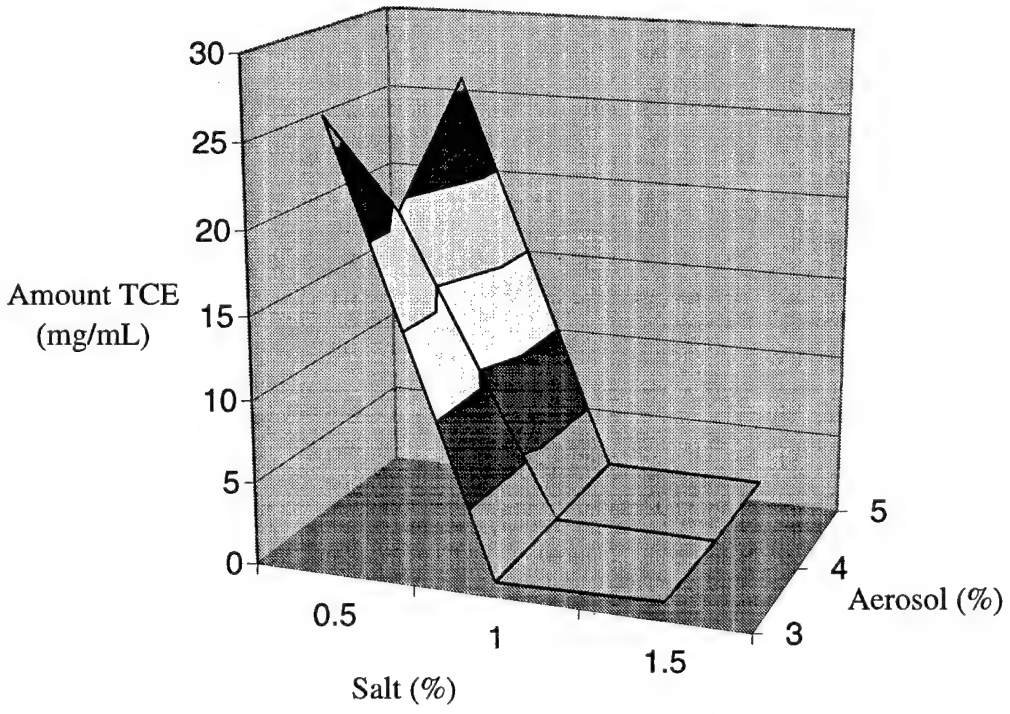


Figure 3.46: Phase Diagram for TCE in 2:1 Aerosol:Isopropanol in OU12 at 10 °C

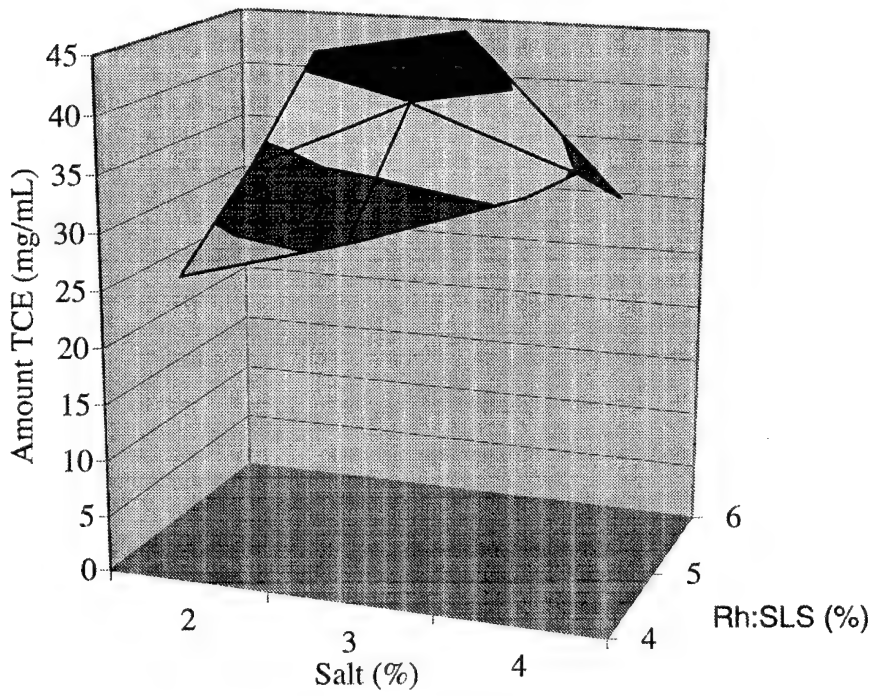


Figure 3.47: Phase Diagram for TCE in 6:1 Rh/SLS:Isopropanol in OU1 at 10 °C

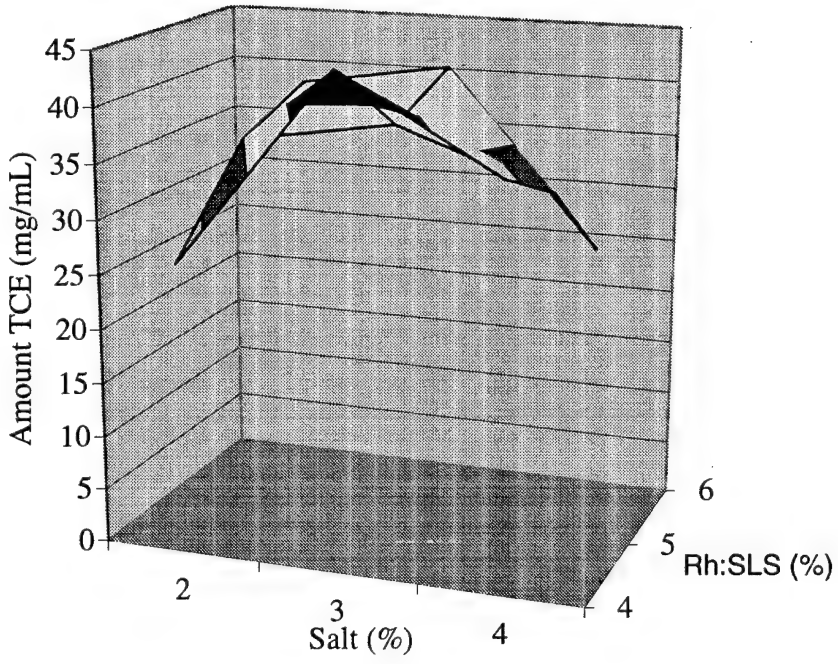


Figure 3.48: Phase Diagram for TCE in 6:1 Rh/SLS:Isopropanol in OU2 at 10 °C

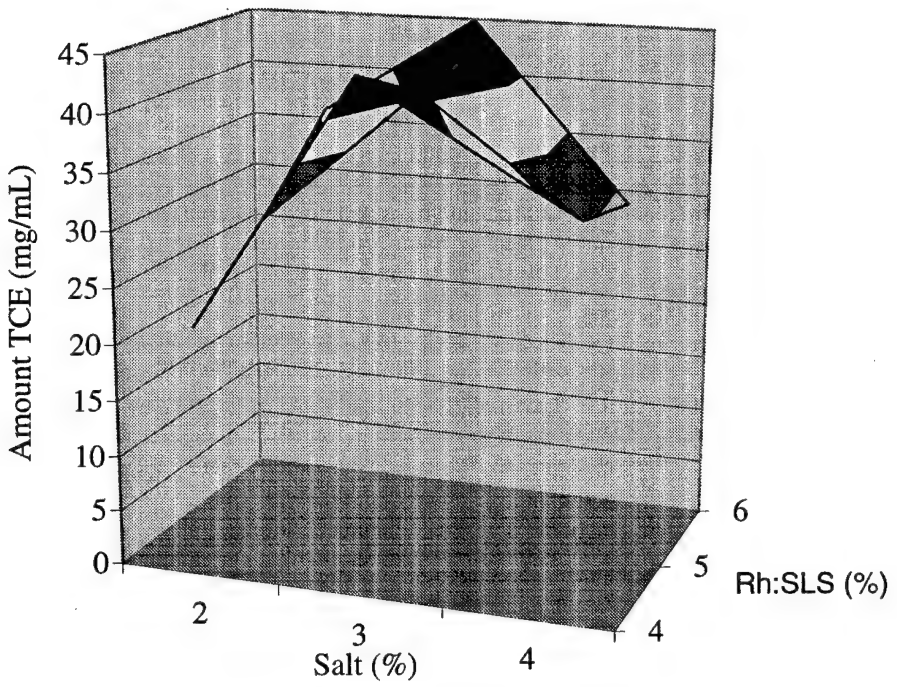


Figure 3.49: Phase Diagram for TCE in 6:1 Rh/SLS:Isopropanol in OU5 at 10 °C

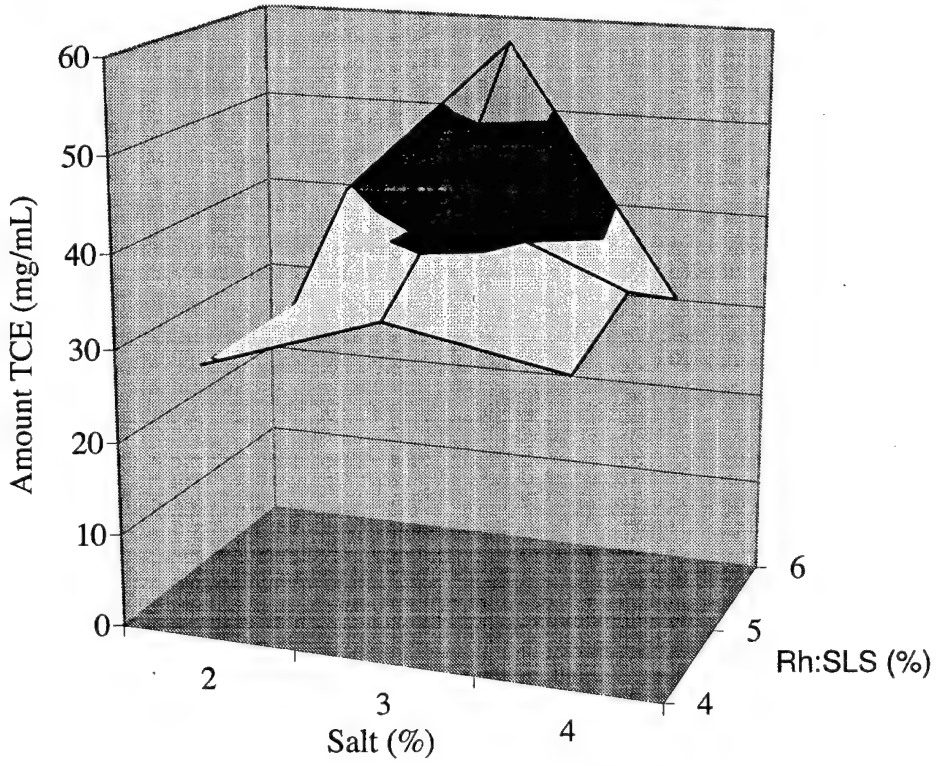


Figure 3.50: Phase Diagram for TCE in 6:1 Rh/SLS:Isopropanol in OU12 at 10 °C

From the phase diagrams, the optimum surfactant mixtures were determined mainly as the surfactant composition (% surfactant, % salt, and % isopropanol) that gives the maximum amount of TCE in mg/mL. Tables 3.4 – 3.6 below give the various amounts of TCE in the emulsion or microemulsion layer for each surfactant composition.

Table 3.4: Amount of TCE (mg/mL) in Various Surfactant Compositions Using Rhema

	OU1				OU5		
	2% salt	3% salt	4% salt		2% salt	3% salt	4% salt
3% Rhema	3.5	2.8	2.9	3% Rhema	2.0	1.7	2.5
4% Rhema	4.5	5.2	3.4	4% Rhema	3.0	4.2	4.1
5% Rhema	5.2	6.1	4.3	5% Rhema	5.3	6.3	9.3
	OU2				OU12		
	2% salt	3% salt	4% salt		2% salt	3% salt	4% salt
3% Rhema	3.0	2.3	3.2	3% Rhema	4.5	4.6	4.7
4% Rhema	1.3	4.5	3.8	4% Rhema	5.2	4.3	7.8
5% Rhema	5.3	7.0	5.7	5% Rhema	5.7	6.3	6.3

Table 3.5: Amount of TCE (mg/mL) in Various Surfactant Compositions Using Aerosol

	OU1				OU5		
	0.5% salt	1% salt	1.5% salt		0.5% salt	1% salt	1.5% salt
3% Aerosol	1.3	1.0	0.8	3% Aerosol	35.9	23.6	1.2
4% Aerosol	15.1	0.9	0.8	4% Aerosol	9.7	1.2	1.3
5% Aerosol	20.3	0.9	0.8	5% Aerosol	52.1	1.1	1.3
	OU2				OU12		
	0.5% salt	1% salt	1.5% salt		0.5% salt	1% salt	1.5% salt
3% Aerosol	18.6	1.0	1.0	3% Aerosol	26.8	0.9	1.2
4% Aerosol	24.9	1.3	1.2	4% Aerosol	19.3	1.1	1.1
5% Aerosol	20.1	1.1	1.3	5% Aerosol	25.8	1.4	1.3

Table 3.6: Amount of TCE (mg/mL) in Various Surfactant Compositions Using 6: 1 Rhema:SLS Blend

	OU1				OU5		
	2% salt	3% salt	4% salt		2% salt	3% salt	4% salt
4% Rh:SLS	27.0	30.9	35.8	4% Rh:SLS	22.3	44.5	36.3
5% Rh:SLS	33.4	39.9	34.8	5% Rh:SLS	28.3	40.7	30.5
6% Rh:SLS	41.4	44.0	29.5	6% Rh:SLS	35.8	44.9	28.8
	OU2				OU12		
	2% salt	3% salt	4% salt		2% salt	3% salt	4% salt
4% Rh:SLS	26.7	44.6	36.4	4% Rh:SLS	29.4	35.7	32.1
5% Rh:SLS	35.0	37.2	32.1	5% Rh:SLS	27.6	43.3	35.6
6% Rh:SLS	37.9	40.1	23.5	6% Rh:SLS	39.4	57.3	30.0

The optimum surfactant compositions of the various surfactant mixtures are summarized in Table 3.7. The selected optimum surfactant compositions are currently being used to perform column extractions of various Utah soils

spiked with 1 mg/kg TCE. The columns have been constructed and the hydraulic analysis of the soils completed.

Table 3.7: Optimum surfactant composition selected for column extraction.

Rhema	% Surf	% Isoprop	% Salt
OU1	5	1	3
OU2	5	1	3
OU5	5	1	4
OU12	5	1	3
Aerosol	% Surf	% Isoprop	% Salt
OU1	5	1	0.5
OU2	5	1	0.5
OU5	5	1	0.5
OU12	5	1	0.5
Rhema/SLS	% Surf	% Isoprop	% Salt
OU1	6	1	3
OU2	6	1	3
OU5	6	1	3
OU12	6	1	3

3.5 Shake Vial Tests

This work focused on the recovery of TCE from spiked soil samples using three surfactant solutions described above. The test soils were OU1, OU2, OU5, and OU12 spiked with 1,000 mg/kg TCE. The three surfactant solutions were prepared in the waters from each site based on previously conducted optimization studies. After preparation, the surfactant samples were chilled to 50° F and filtered to remove precipitated material that formed from contact with the amendments and the site waters.

- Aerosol (2: 1) ratio serfactant:isopropanol, 0.5% salt with all waters
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 3% salt in OU1, OU2, OU12
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 4% salt in OU5
- Rhema/SLS (6:1) with (2:1) ratio surfactant: isopropanol, 3% salt in all waters

Tests were performed with two similar methods. Results from the first method led us to suspect that volatilization of TCE might have been a problem in the procedure, so the tests were repeated with Method 2 designed to reduce the potential for volatile losses. Results from Method 2 were similar to Method 1, so both sets are presented.

Recovery of TCE from the soils varied marginally by the surfactant used, with Rhema Super Matrix giving slightly higher results. Overall, recovery was between 8 and 15% of the total initial TCE spike.

Method 1: Soil (10 g) was placed into a screw cap vial. TCE in the amount of 6.8 µL (9.9 mg) was added to the soil sample and the screw cap was closed. The vial was shaken to disperse the TCE. Surfactant (15 mL) was then added to the vial and the contents reshaken. The vial was then placed into a 50 °F chamber for a few days. A 10 mL volume of surfactant was recovered and placed with 7 g salt into a GC headspace vial. The headspace vial was then capped and the contents analyzed. All samples were run in triplicate.

Method 2: A plastic sheet was used to construct a confined atmospheric space. A beaker of TCE was placed into the confined atmosphere and the atmosphere was allowed to equilibrate with the TCE. All further manipulations were performed within the saturated atmosphere. Two sets of samples were prepared, spiked samples and atmospheric controls. The spiked samples of soil (10 g) were prepared as follows in GC head space vials. Soil was placed into the vial and TCE in the amount of 7 µL (10.2 mg) was added. The vial was shaken to disperse the TCE. Surfactant (29-32 mL) was then added to completely fill the vial to eliminate headspace. The vial was capped and crimped, and the contents reshaken. After the contents of the vial settled, a 10 mL volume of surfactant was recovered and placed with 7 g salt into a GC headspace vial. The headspace vial was then capped and the contents analyzed. Controls were prepared by the same method except that no additional spike was added to the soil. Recovery of TCE from the controls represented the small amount of TCE that was potentially adsorbed from contact with atmospheric TCE vapor. All samples were run in triplicate.

Recovery results for the spiked soil shake tests using Method 1 are summarized in Tables 3.8 – 3.10. Figures 3.51 and 3.52 show the removal of TCE from soils (on concentration and % recover basis) using Method 1. Results with Method 2 are presented in Tables 3.11 – 3.13. Results are presented as the concentration of TCE in the recovered surfactant and as % recovery calculated as the product of the concentration in the recovered surfactant and the total volume of surfactant used. Figures 3.53 and 3.54 show the removal of TCE from soils on a concentration basis using Method 2. Figure 3.55 is a summary of results from the two test methods analyzed together on a concentration basis. The larger standard deviations for analysis of the two sets together reflects the differences between the two sets. In general, Rhema and Rhema/SLS performed better than aerosol, however, a detailed statisitcal analysis on this trend has not been performed.

Table 3.8: Method 1: Recovery of TCE from Soils Using Rhema (Shake Tests)							
Soil	mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	9.95	0.71	0.24	0.05	0.02	7.2	2.9
OU2	9.95	0.53	0.14	0.04	0.01	5.4	1.8
OU5	9.95	1.71	0.17	0.11	0.01	17.1	2.1
OU12	9.95	0.72	0.10	0.05	0.01	7.3	1.2

Table 3.9: Method 1: Recovery of TCE from Soils Using Aerosol (Shake Tests)							
Soil	mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	9.95	0.37	0.04	0.02	0.00	3.7	0.4
OU2	9.95	0.35	0.16	0.02	0.01	3.5	2.2
OU5	9.95	0.64	0.13	0.04	0.01	6.4	1.6
OU12	9.95	0.40	0.17	0.03	0.01	4.0	2.0

Figure 3.51: TCE Recovery for Batch Extraction of Utah Soils
Method 1

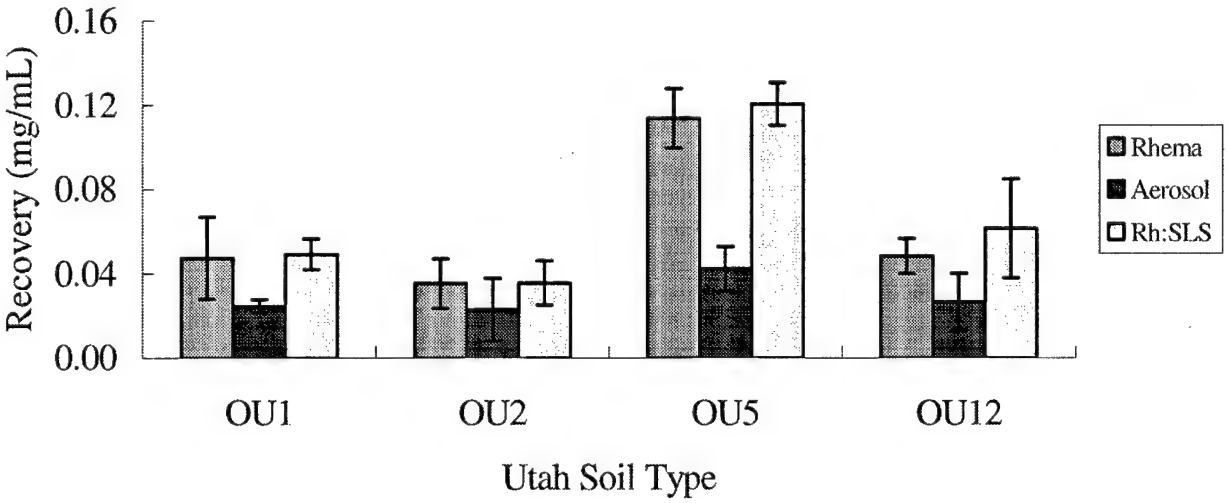


Table 3.10: Method 1: Recovery of TCE from Soils Using Rhema/SLS (Shake Tests)							
Soil	mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	9.95	0.74	0.09	0.05	0.01	7.4	1.1
OU2	9.95	0.54	0.13	0.04	0.01	5.4	1.6
OU5	9.95	1.81	0.13	0.12	0.01	18.2	1.5
OU12	9.95	0.92	0.29	0.06	0.02	9.2	3.5

Figure 3.52: Percent TCE Recovery for Batch Extraction of Utah Soils
Method 1

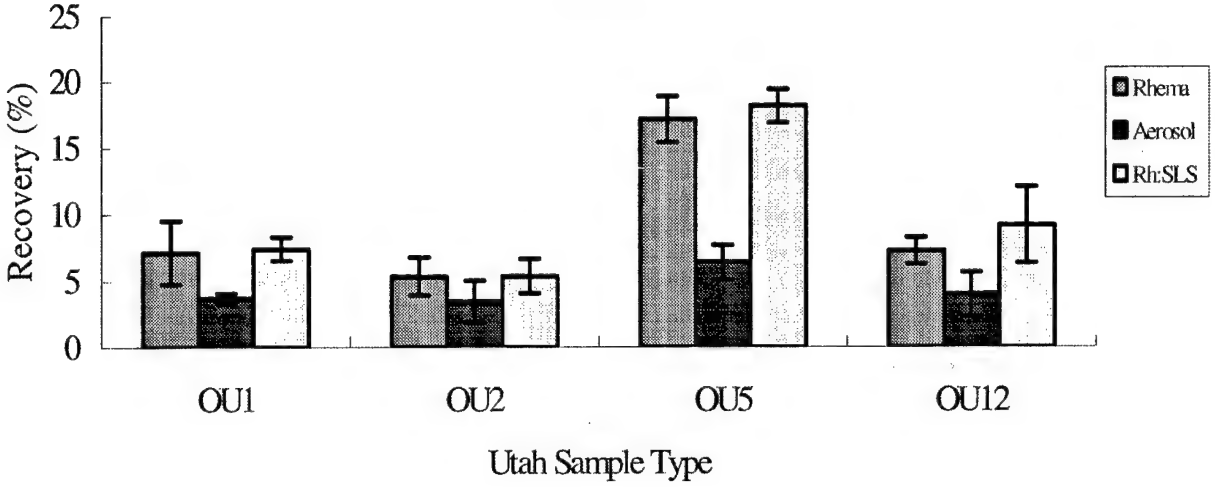


Table 3.11: Method 2: Recovery of TCE from Soils Using Rhema (Shake Tests)

Soil	mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	10.0	0.90	0.18	0.03	0.01	8.7	1.8
OU2	10.0	1.94	0.33	0.06	0.01	18.7	3.2
OU5	10.0	2.74	0.24	0.09	0.01	26.5	2.3
OU12	10.0	0.98	0.08	0.03	0.00	9.5	0.8

Table 3.12: Method 2: Recovery of TCE from Soils Using Aerosol (Shake Tests)

Soil	Mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	10.0	1.14	0.29	0.04	0.01	11.1	2.8
OU2	10.0	1.64	0.40	0.06	0.01	16.0	3.9
OU5	10.0	1.87	0.16	0.06	0.01	18.1	1.5
OU12	10.0	0.80	0.21	0.03	0.01	7.8	2.1

Table 3.13: Method 2: Recovery of TCE from Soils Using Rhema/SLS (Shake Tests)

Soil	mg Used	mg Recov	S.D.	mg/mL rec	S.D.	% Recov	S.D.
OU1	10.0	0.66	0.32	0.02	0.01	6.4	3.1
OU2	10.0	1.28	0.23	0.04	0.01	12.3	2.2
OU5	10.0	1.46	0.36	0.05	0.01	14.1	3.4
OU12	10.0	0.51	0.37	0.02	0.00	7.0	0.8

Figure 3.53: TCE Recovery for Batch Extraction of Utah Soils
(Method 2, TCE Atmosphere, Sample-in-a-Bag)

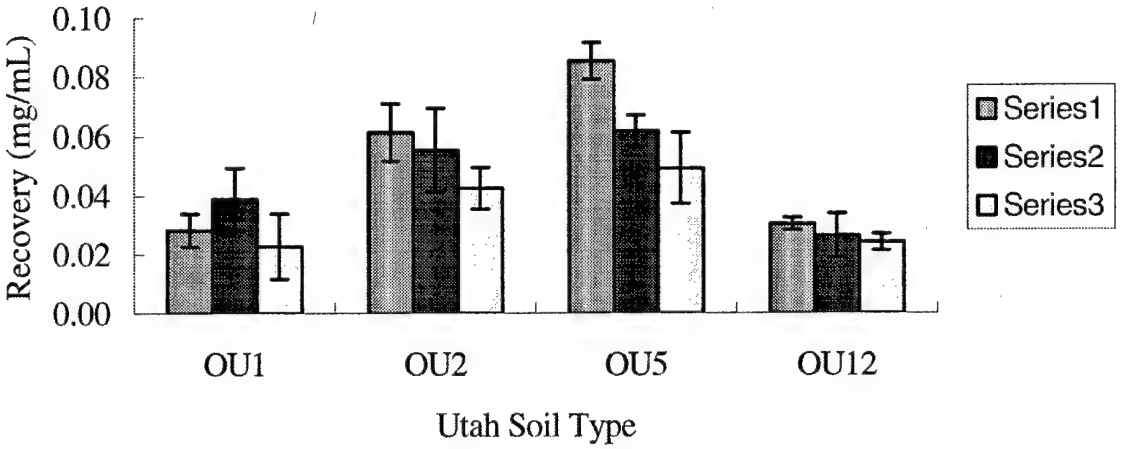


Figure 3.54: Percent TCE Recovery for Batch Extraction of
Utah Soils (Sample-in-a-Bag) Method 2

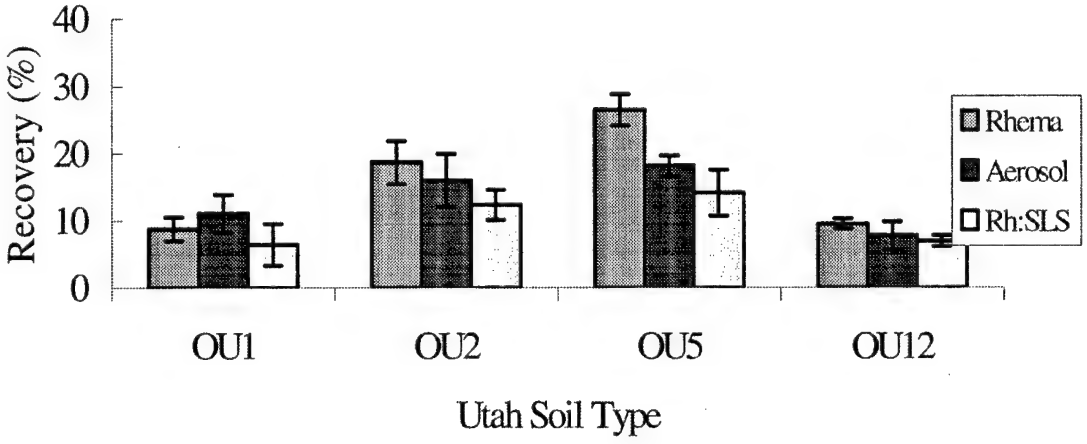
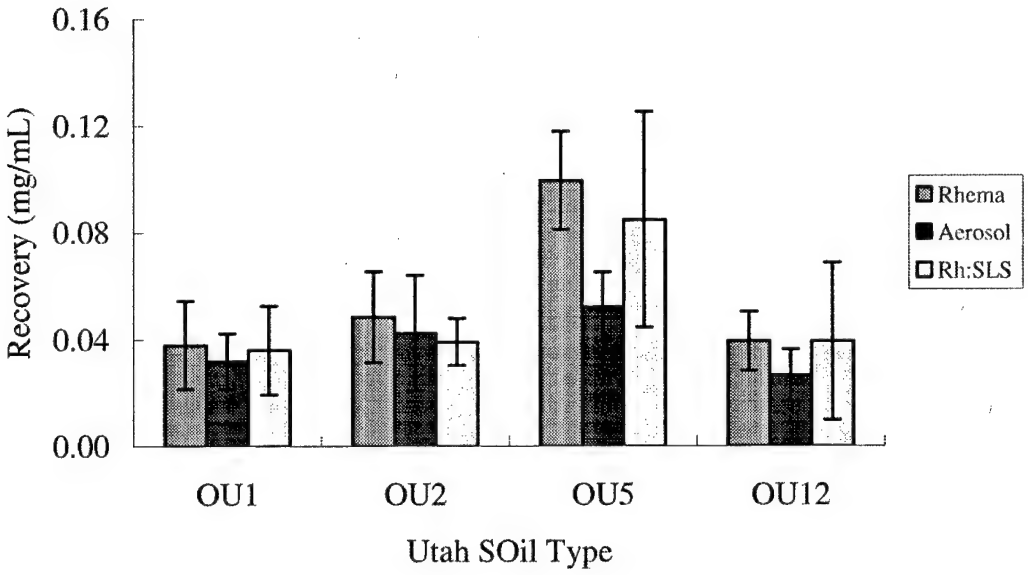


Figure 3.55: TCE Recovery for Batch Extraction
Combined data sets, Methods 1 and 2



3.6 Soil Column Hydraulics

The soil column design was finalized as depicted in Figure 2.3. The apparatus consisted of a 2 L separatory funnel used as a surfactant reservoir suspended above the column. The flow inlet to the column was located at the base of the column. A full-port cylinder valve controlled the flow. The details of the column internals are presented in the next paragraph. The exit from the column is at the top of the unit. A flow stop valve is located just prior to the discharge line. The discharge line flowed into a 2 L separatory funnel used as the collection vessel for spent surfactant. The available head in the system was between 90 and 100 cm water, representing the high and low fluid levels in the inlet reservoir. During hydraulic capacity testing, flow was maintained constant by periodically replacing fluid in the reservoir at some mid-point between high and low volume. The target fluctuation in the reservoir level was less than 5 cm. During tests with surfactant and contaminated soil, the reservoir was filled with 1.5 L surfactant and allowed to fully drain through the column such that about 8 volume displacements were achieved through the soil. All tests were run in a controlled environmental chamber set at 10°C to simulate groundwater temperature conditions.

Hydraulic analysis of the columns was performed using a modified Darcy equation for flow through a porous medium (Freeze and Cherry, 1979, Severin and Grethlein, 1996). The flow equation follows (see section 3) a series resistance law for steady state flow in which the system losses

(column, tubing, piping, etc without packed soil) are analyzed and subtracted independently from the total losses (column and piping plus packed soil).

The system hydraulic performance was measured with all equipment in place, but with no soil in the column. Test data are shown in Table 3.14. The area of the column, A, is 19.6 cm^2 and the soil depth, L, is 10 cm. The volume of water, V, passed through the system during time period T is equal to the flow, Q. The virtual system conductivity (system depth without soil) per virtual depth (*k/l*, sec^{-1}) (system depth without soil) was determined to be 1.1 x 10^{-3} sec^{-1}. This value was used to estimate the soil conductivity for each soil type. The soil conductivity for OU1 and OU 12 were moderate and represent soils that are well suited for surfactant flushing technology, being between 2.0 x 10^{-3} and 2.7 x 10^{-3} cm sec^{-1}. Likewise, OU2 is a good candidate soil for *in situ* remediation with a hydraulic conductivity of 1.2 x 10^{-2} cm/sec. The OU5 soil conductivity is marginal, being 5 x 10^{-4} cm/sec, but still within acceptable ranges of conductivity.

Table 3.14: Summary of Hydraulic Conductivity Data in the Packed columns

	V (mL)	T (sec)	h (cm)	Q/Ah (sec^{-1})	k/l (sec^{-1})	K/L (sec^{-1})	K (cm sec^{-1}) conductivity
No Soil	64	30	96	1.1E-03	1.1E-03		
	65	30	97	1.1E-03			
	63	30	95	1.1E-03			
OU1	19.5	60	96	1.7 E-04	1.1E-03	2.0E-04	2.0E-03
	19	60	95	1.7 E-04		2.0E-04	2.0E-03
	18.5	60	94	1.7 E-04		2.0E-04	2.0E-03
OU2	61	30	100	1.0E-03	1.1E-03	1.2E-02	1.2E-01
	59	30	98	1.0E-03		1.1E-02	1.1E-01
	59	30	96	1.0E-03		1.3E-02	1.3E-01
OU5	6	60	97	5.3E-05	1.1E-03	5.5E-05	5.5E-04
	6	60	96	5.3E-05		5.6E-05	5.6E-04
	6	60	96	5.3E-05		5.6E-05	5.6E-04
OU12	18	45	94.5	2.2E-04	1.1E-03	2.7E-04	2.7E-03
	16.5	45	94	2.0E-04		2.4E-04	2.4E-03
	15.5	45	93.6	1.9E-04		2.2E-04	2.2E-03

3.7 Soil Column Extraction Tests

Column extraction tests were performed using two series of tests. In the first series (Method 1), soil was dosed with 1,000 mg/kg TCE and left in capped glass jars for 5-6 weeks prior to testing. Columns were loaded with about 300 g of TCE spiked soil. All soils and solutions were brought to constant temperature (50° F) overnight before testing. Some of the test waters reacted with the salt:surfactant:alcohol solution to produce precipitates. No attempt was made to alter the condition of the surfactant solutions. Column extractions were performed at 50° F in a temperature controlled room. A 250 ml salt pretreatment was followed by surfactant treatment. Flow was by gravity drainage. Pretreatment fluid and surfactant were collected from the

column into open flasks. Samples were collected for analysis after the salt flush and then periodically during the surfactant run. Some tests had limited percolation of surfactant and not all of the full 1 L dose of surfactant could be run through some of the columns. All columns were stopped after 16 hours drainage time. Summary results for the tests with Method 1 are presented in Table 4.15. Tests that had limited percolation of surfactant are labeled in bold. These are indicated for any test that passed less than 150 ml of the 250 ml presalt solution, and less than 900 ml of the 1,000 ml surfactant solution.

Low recoveries of TCE brought a concern that performing the test procedures at atmospheric conditions limited the recovery of TCE in the surfactant, probably due to evaporation. In Method 2, a plastic sheet was used to construct a limited exposure atmosphere. A beaker of TCE was placed within the plastic enclosure and allowed to equilibrate to create a TCE-saturated atmosphere and all soils were spiked in the controlled atmosphere chamber after equilibrating for at least five hours. All soils and solutions were brought to temperature (50° F) overnight before loading into the columns for extraction. Some of the site waters reacted with some of the surfactant:salt:alcohol solutions to form precipitates. Precipitates were filtered from those waters on a No.1 Wattman filter prior to application of the fluid to the columns. Column extractions were performed in a 50° F controlled temperature room. A 250 ml salt pretreatment preceded each surfactant treatment. Flow was by gravity drainage. Pretreatment fluid and surfactant were collected separately from the column into 1.2 L Tedlar bags to limit volatile losses. Salt prewash and surfactant were analyzed separately. Results for Method 2 are presented in Table 3.16. Tests that had limited percolation of surfactant are labeled in bold. These are indicated for any test that passed less than 150 mL of the 250 mL presalt solution and less than 900 mL of the 1,000 mL surfactant solution originally applied.

In both series of tests, water from each site was prepared with a pre-chosen concentration of surfactant, salt, and isopropanol. These were the same as used in the shake vial tests, with the exception that by the second series of tests, we had used all the available OU5 water. The second set of OU5 column tests was prepared using Lansing, Michigan tap water.

- Aerosol (2:1) ratio of surfactant:isopropanol, 0.5% salt with all waters
- Rhema Super Matrix (1:1) ratio of surfactant:isopropanol, 3% salt in OU1, OU2, OU12
- Rhema Super Matrix (1:1) ratio of surfactant:isopropanol, 4% salt in OU5
- Rhema/SLS (6:1) with (2:1) ratio of surfactant:isopropanol, 3% salt in all waters
- OU5 surfactant in the second set was prepared with Lansing Michigan tap water.

Table 3.15 shows the results of column extractions of TCE from various TCE-spiked Utah soils (labeled OU1-OU12) using different surfactant mixtures in Utah waters. Rhema and Rhema:SLS surfactant mixtures visibly contained precipitates at the extraction temperature of 50° F resulting in flow problems. As a result, out of eight column extractions using Rhema and Rhema:SLS mixtures, only two resulted in complete surfactant flow through the soil columns. On the other hand, aerosol easily flowed through the soil columns with almost 100% surfactant recovery. However, the amount of TCE extracted appears to be independent of the amount of surfactant that passed through the soil column. Overall, 0.08 – 2.2% TCE was recovered in 3 out of 12 extractions. In all other tests, the amount of TCE extracted was not detectable. This low or no recovery for all samples was thought to be, possibly, a result of loss of TCE through volatilization before extractions.

To minimize volatilization of the TCE before sample extraction, the soil samples were spiked and packed in a TCE-saturated atmosphere. Since the atmosphere was saturated with TCE, a negligible amount of the spiked TCE was expected to volatilize. In addition, all surfactant solutions that had precipitates were filtered to improve flow through the soil columns. Table 3.16 shows results of column extractions of soil samples spiked and packed in the TCE atmosphere. There was improved column flow in all but 3 column extractions as about 90% or more of all surfactants flowed through the columns. Table 4.17 showed that neither the TCE atmosphere nor the amount of surfactant through the sample improved TCE recoveries as only 0.02 – 1.78% recoveries were recorded. However, almost three times the number of samples recorded detectable levels of TCE, but it was inconclusive as to whether this was a result of the conditions used or higher than expected initial TCE due to adsorbed TCE from the TCE-saturated atmosphere.

In conclusion, all the surfactants appear to be ineffective in extracting TCE from column samples. It is, however, possible that changes in experimental conditions such as flow rates, column packing, and surfactant composition would help improve TCE recoveries. Detailed information about these data sets is found in Tables 3.17 – 3.22.

Table 3.15: Summary of Column Extractions With Utah Soils:
Method 1: Full Atmospheric Exposure

Rhema	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	N.D	150	0.36	150	N.D	150	0.08	150
Rhema	N.D	500	1.85	1000	N.D	200	N.D	450
Total	N.D		2.21		N.D		0.08	
Aerosol	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	N.D	150	0.18	150	N.D	150	N.D	150
Aerosol	N.D	800	0.31	1000	N.D	1000	N.D	1000
Total	N.D		0.49		N.D		N.D	
Rh:SLS	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	N.D	134	N.D	102	N.D	105	N.D	132
Rh:SLS	N.D	415	N.D	152	N.D	1000	N.D	90
Total	N.D		N.D		N.D		N.D	

Bold indicates incomplete passage of solution, less than 150 ml presalt or 900 ml surfactant passed.
N.D. equals non-detection of TCE in recovered samples.

Table 3.16: Summary of Column Extractions In Utah Soils:
Method 2, Limited Atmospheric Exposure

Rhema	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	0.62	172	0.05	122	0.13	94	0.05	170
Rhema	1.03	1040	0.37	100	2.07	930	N.D	150
Total	1.65		0.42		2.2		0.05	
Aerosol	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	N.D	132	0.04	40	0.02	38	N.D	30
Aerosol	N.D	940	1.74	960	N.D	900	N.D	960
Total	N.D		1.78		0.02		N.D	
Rh:SLS	OU1 % Recovered	Volume Extracted	OU2 % Recovered	Volume Extracted	OU5 % Recovered	Volume Extracted	OU12 % Recovered	Volume Extracted
Presalt	0.12	152	0.1	149	0.12	112	N.D	40
Rh:SLS	N.D	990	0.38	890	N.D	200	N.D	860
Total	0.12		0.48		0.12		N.D	
Total	0.12		0.48		0.12		N.D	

Bold indicates incomplete passage of solution, less than 150 ml presalt or 900 ml surfactant passed.
N.D. equals non-detection of TCE in recovered samples.

No reference is made in the text to Tables 3.17 – 3.22. These represent the detailed results while Tables 3.15 – 3.16 are the summaries.

Table 3.17: Column Extraction with 1:1 Rhema:Iso @ 10 deg C in Utah Soil
Method 1: Full Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10mL	mg recov/vol used	% extracted
OU1								
presalt	150			10	N.D	N.D	N.D	N.D
200 mL	200			10	N.D	N.D	N.D	N.D
300 mL	300	294	294	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D
OU2								
presalt	150			10	24237	0.07	1.06	0.36
200 mL	200			10	24940	0.07	1.45	0.49
400 mL	400			10	15289	0.05	1.83	0.62
1000 mL	1000	293	293	10	18343	0.05	5.41	1.85
						Total	6.47	2.21
OU5								
presalt	150			10	N.D	N.D	N.D	N.D
200 mL	200	284	284	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D
OU12								
presalt	150			10	5083	0.02	0.26	0.08
200 mL	200			10	N.D	N.D	N.D	N.D
250 mL	250	309	309	10	N.D	N.D	N.D	N.D
						Total	0.26	0.08%

Table 3.18: Column Extraction with 2:1 Aerosol:Iso @ 10 deg C in Utah Soil
Method 1: Full Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10mL	mg recov/vol used	% extracted
OU12								
presalt	150			10	N.D	N.D	N.D	N.D
200 mL	200			10	N.D	N.D	N.D	N.D
400 mL	400			10	N.D	N.D	N.D	N.D
600 mL	600			10	N.D	N.D	N.D	N.D
888 mL	800	389	389	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D
OU2								
presalt	150			10	12229	0.04	0.54	0.18
200 mL	200			10	8055.5	0.02	0.50	0.16
400 mL	400			10	7357.5	0.02	0.92	0.30
600 mL	600			10	5382	0.02	1.06	0.35
800 mL	800			10	6170	0.02	1.58	0.52
1000 mL	1000	301	301	10	2324	0.01	0.93	0.31
						Total	1.48	0.49
OU5								
presalt	150			10	N.D	N.D	N.D	N.D
200 mL	200			10	N.D	N.D	N.D	N.D
	400			10	N.D	N.D	N.D	N.D
	600			10	N.D	N.D	N.D	N.D
	800			10	N.D	N.D	N.D	N.D
	1000	291	291	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D
OU12								
presalt	150			10	N.D	N.D	N.D	N.D
200 mL	200			10	N.D	N.D	N.D	N.D
250 mL	400			10	N.D	N.D	N.D	N.D
	600			10	N.D	N.D	N.D	N.D
	800			10	N.D	N.D	N.D	N.D
	1000	303	303	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D

Table 3.19: Column Extraction with 6:1 Rhema:SLS @ 10 deg C in Utah Water
Method 1: Full Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10 mL	mg recov/vol used	% extracted
OU1								
presalt	134			10	N.D	N.D	N.D	N.D
1	200			10	N.D	N.D	N.D	N.D
2	215	303	294	10	N.D	N.D	N.D	N.D
						Total	N.D	N.D
OU2								
presalt	102			10	N.D	N.D	N.D	N.D
1	152	295	295	10	N.D	N.D	N.D	N.D
						Final	N.D	N.D
OU5								
presalt	105			10	N.D	N.D	N.D	N.D
1	200			10	N.D	N.D	N.D	N.D
2	400			10	N.D	N.D	N.D	N.D
3	1000	250	250	10	N.D	N.D	N.D	N.D
						Final	N.D	N.D
OU12								
presalt	132			10	N.D	N.D	N.D	N.D
1	90	261	261	10	N.D	N.D	N.D	N.D
						Final	N.D	N.D

Table 3.20: Column Extraction with 1:1 Rhema:Iso @ 10 deg C in Utah Soil
Method 2: Limited Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10 mL	mg recov/vol used	% extracted
				OU1				
presalt	172			10	57864	0.11	1.84	0.62
13	1040	295	295	10	13923	0.03	3.02	1.02
						Total	4.86	1.65
				OU2				
presalt	122			10	4714	0.01	0.16	0.05
14	100	296	296	10	59367	0.11	1.09	0.37
						Final	1.25	0.42
				OU5				
presalt	94			10	19371	0.04	0.36	0.13
15	930	275	275	10	32012	0.06	5.68	2.06
						Final	6.04	2.20
				OU12				
presalt	170			10	2865	0.01	0.16	0.05
16	150	306	306	10	0	N.D	N.D	N.D
						Final	0.16	0.05

Table 3.21: Column Extraction with 2:1 Aerosol:Iso @ 10 deg C in Utah Soil
Method 2: Limited Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10 mL	mg recov/vol used	% extracted
				OU1				
presalt	132			10	0	N.D	N.D	N.D
17	940	295	295	10	0	N.D	N.D	N.D
						Total	N.D	N.D
				OU2				
presalt	40			10	12786	0.03	0.11	0.04
18	960	296	296	10	27855	0.05	5.15	1.74
						Final	5.26	1.78
				OU5				
presalt	38			10	4579	0.01	0.05	0.02
19	900	275	275	10	0	N.D	N.D	N.D
						Final	0.05	0.02
				OU12				
presalt	30			10	0	N.D	N.D	N.D
20	960	306	306	10	0	N.D	N.D	N.D
						Final	N.D	N.D

Table 3.22: Column Extraction with 6:1 Rhema:SLS @ 10 deg C in Utah Soil
Method 2: Limited Atmospheric Exposure

Sample #	Amt Surf	g soil used	mg/wt soil used	Vol Used	Peak Area	mg recov/10 mL	mg recov/vol used	% extracted
				OU1				
presalt	152			10	10307	0.02	0.34	0.12
21	990	295	295	10	0	N.D	N.D	N.D
						Total	0.34	0.12
				OU2				
presalt	149			10	8897	0.02	0.30	0.10
22	890	296	296	10	4595.5	0.01	1.12	0.38
						Total	1.42	0.48
				OU5				
presalt	112			10	13864.5	0.03	0.32	0.12
23	200	275	275	10	0	N.D	N.D	N.D
						Total	0.32	0.12
				OU12				
presalt	40			10	0	N.D	N.D	N.D
24	860	306	306	10	0	N.D	N.D	N.D
						Total	N.D	N.D

4. Conclusions

The objective of this project was to evaluate surfactant systems for potential use in surfactant flushing of TCE from various soil types at Hill AFB. Soils and waters from four sites were evaluated and included OU1, OU2, OU5, and OU12.

Six surfactants were tested. The surfactants included an alkaline cleanser Rhema Super Matrix; three anionic surfactants, sodium lauryl sulfate, Dowfax, and aerosol, and two non-ionic surfactants, Witconol and Triton. As the various surfactants were removed from study at the various screening levels, a blend of Rhema Super Matrix and SLS was created that has some interesting temperature stability properties.

The screening was performed in the following manner, in an attempt to cull products that did not appear to be effective. The experimental design followed closely to the objectives outlined in the work plan (Statement of Work). More specifically, the tests were designed to:

1. Propose a series of surfactants with potential for success.
2. Challenge the surfactants with soluble calcium to cull those with strong reactivity.
3. Optimize the remaining surfactants to carry TCE in soluble micro-emulsion phase.
4. Establish the optimum surfactant range for the remaining surfactants using site waters.
5. Remove TCE from spiked site soils (1000 mg/kg spike) in shake vial equilibrium tests.
6. Remove TCE from spiked site soils (1000 mg/kb spike) in flow-through column studies.

Conclusions from the initial screening tests indicated that:

Table 4.1: Calcium Interference Tests in OU5 Water

Surfactant	OU5 Water	OU5 + Isoprpanol	OU5+Isopropanol + Calcium chloride
Aerosol	Desirable, up to 20% surfactant	Desirable, up to 20% surfactant	Acceptable, but loss of TCE retention with increased calcium
Rhema	Acceptable up to 10% surfactant	Acceptable up to 25% surfactant	Unacceptable phase splits and precipitation
Dowfax	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant	Acceptable up to 5 % surfactant
Witconol	Unacceptable at all concentrations	Unacceptable at all concentrations	Unacceptable, all concentrations
SLS	Acceptable at all concentration	Acceptable at all concentration	Unacceptable at all concentrations, gel formation
Triton	Acceptable at all concentration	Acceptable at all concentration	Hazy, no clear indication of capacity for TCE mobilization

- Aerosol initially showed a large solubilization of TCE into the water phase (Figure 4.1) and retained about the same degree of solubilization with the addition of isopropanol (Figure 4.2). Upon addition of calcium carbonate, the water released the excess TCE (Figure 4.3).
- Rhema has acceptable behavior with doses up to 10% surfactant. Thereafter, the surfactant split into several complex phases (Figure 4.4). With added isopropanol, the complex phase splits were lessened and retarded until 25% surfactant (Figure 4.5). In the presence of heavy calcium brine, Rhema was completely unacceptable with heavy precipitation within the TCE layer (Figure 4.6).
- Dowfax was acceptable up to 5% in OU5 water, but generated a precipitated surfactant layer at the TCE-water interface at higher concentrations (Figure 4.7). Addition of isopropanol resulted in increased precipitation (Figure 4.8), with slight improvement with added brine (Figure 4.9).
- Witconol was unacceptable at all concentration ranges in OU5 water, with three to four complex phases generated (Figure 4.10). Slight improvements were seen with isopropanol (Figure 4.11) and calcium additions (Figure 4.12). However, heavy precipitation of the surfactant rendered this surfactant completely unacceptable.
- SLS was acceptable at all concentrations up to 20% in water (Figure 4.13) and isopropanol (Figure 4.14), but was susceptible to precipitation and gel formation in the TCE layer with added calcium (Figure 4.15).
- Triton formed a hazy intermediate layer amounting to about 4% of the sample volumes (Figures 4.16, 4.17, 4.18).

Based on this screening, MBI recommends elimination of Dowfax, Witconol and Triton from further testing. SLS will be used with limited brine, and Rhema will be used in concentrations less than 10%.

The next screening test was to generate micro-emulsions to carry the TCE in the water phase. Screening tests continued to identify suitable ranges of surfactant in Lansing tap water. In these tests, isopropanol was used as a co-solvent in the ratio of 1:1 and 2:1 mass units of surfactant to ispropanol. Sodium chloride was used as surfactant desensitizer. Aerosol, SLS, and Rhema were used as the test surfactants. Tests were first performed at room temperature, and then the vials were re-shaken for tests at 10° C.

- Aerosol established an iridescent blue-pearl haze within the water phase at room temperature at all surfactant concentrations from 3 through 6% at a constant salt dose of 1% NaCl. At 10° C, the blue iridescent haze disappeared from the water phase and became established within the TCE layer.
- Rhema produced a rich cloudy-white emulsion within the water layer that disappeared at room temperature over the course of 2-3 days, indicating a true emulsion rather than a micro-emulsion. However, at 10°C, the

cloudy-white emulsion remained stable for well over one week. The emulsion seemed more robust at higher salt and higher surfactant concentrations.

- SLS had a similar response to temperature, as did aerosol. At room temperature, SLS produced a clear microemulsion that could be seen as a break in the refractive index between the water phase and the microemulsion at lower salt concentrations. As the salt increased, the microemulsion became light blue to hazy, and more distinct. As the temperature was decreased to 10° C, the emulsion began to disappear, becoming non-existent at lower salt and surfactant concentrations.

Another goal was to determine the best aqueous surfactant/isopropanol/salt systems that offer maximum extraction capacity of TCE from water. This was done by either contacting 2 g of TCE with 20 g surfactant mixture or 10 g TCE with 10 g surfactant mixture. In all cases, the amount of TCE used was large enough to give saturated surfactant systems upon thorough mixing. The TCE-saturated surfactant mixtures were allowed to equilibrate for at least 48 hours before analysis. When a series of Rhema:SLS mixtures were blended, the TCE carrying capacity at 10° C was vastly improved over straight SLS, Rhema, or aerosol, Figure 4.1. The mixed blends seemed to be unaffected by temperature in the range of 4-15° C.

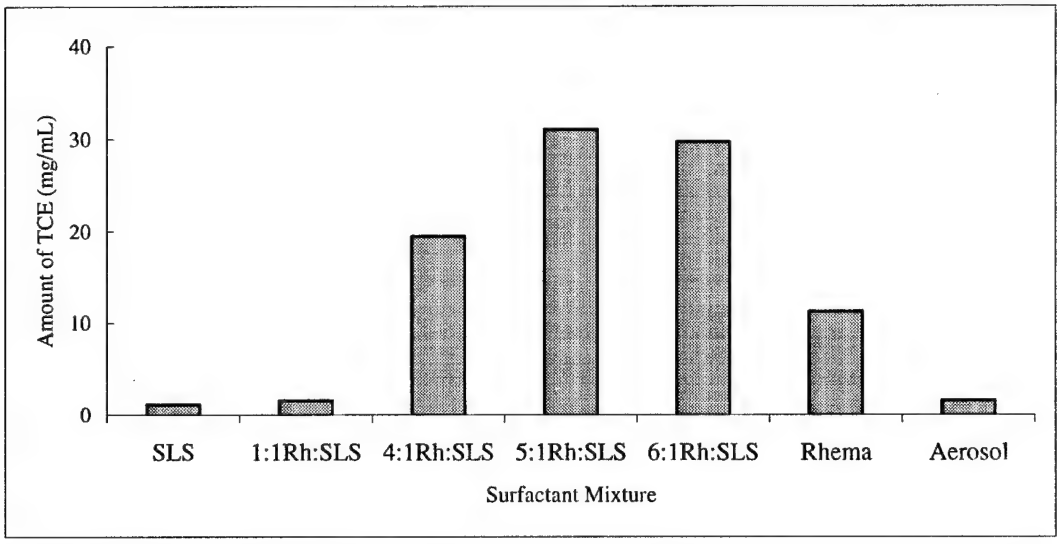


Figure 4.1: Summary of TCE Carrying Capacity of Various Surfactant Mixtures at 10 °C

The optimum surfactant/isopropanol/salt mixtures for Rhema and SLS have been established in Lansing Tap Water to be 5% surfactant, 1:1 surfactant:isopropanol, and 3% salt while that for aerosol is 5% surfactant, 2:1 surfactant:Isopropanol, and 1% salt. With these optimums, we have shown that:

- Rhema carries more TCE than aerosol at all temperatures studied, though Rhema forms an emulsion and SLS forms a clear blue microemulsion.
- Rhema carries more TCE at 10°C than SLS, whereas at room temperature, SLS carries much more TCE and Rhema and aerosol.
- SLS carries very little TCE at 10°C comparable to that of aerosol, but carries very large amounts of TCE at higher temperatures.
- Mixtures of Rhema and SLS tend to increase the amount of TCE carried in the aqueous surfactant solution.
- Higher ratios of Rhema to SLS tend improve the TCE carrying capacities of the aqueous mixtures.

The evaluation of the surfactants Rhema, aerosol, and blended Rhema:SLS in the Utah waters was performed by selecting a range of salt and surfactant concentrations based on the optimum conditions established with Lansing tap water at 10°C. The matrices tested in the Utah water at 10° C were:

- 1:1 Rhema:isopropanol with 3, 4, and 5% surfactant and a range of 2, 3, and 4% sodium chloride.
- 2:1 Aerosol:isopropanol with 3, 4, and 5% surfactant and a range of 0.5, 1, and 1.5% sodium chloride.
- 1:1 Rhema/SLS:ispropanol with the surfactant blend based on 6:1 Rhema:SLS and having a total surfactant concentrations of 4, 5, and 6% and a range of 2, 3, and 4% sodium chloride.

The optimum surfactant compositions of the various surfactant mixtures are summarized in Table 4.2. The selected optimum surfactant compositions are currently being used to perform column extractions of various Utah soils spiked with 1 mg/kg TCE. The columns have been constructed and the hydraulic analysis of the soils completed.

Table 4.2. Optimum surfactant composition selected for column extraction.

Rhema	% Surf	% Isoprop	% Salt
OU1	5	1	3
OU2	5	1	3
OU5	5	1	4
OU12	5	1	3
Aerosol	% Surf	% Isoprop	% Salt
OU1	5	1	0.5
OU2	5	1	0.5
OU5	5	1	0.5
OU12	5	1	0.5
Rhema/SLS	% Surf	% Isoprop	% Salt
OU1	6	1	3
OU2	6	1	3
OU5	6	1	3
OU12	6	1	3

Spiked soils were extracted in shake flasks in single extraction tests. This work focused on the recovery of TCE from spiked soil samples using three surfactant solutions described above. The test soils were OU1, OU2, OU5, and OU12 spiked with 1,000 mg/kg TCE. The three surfactant solutions were prepared in the waters from each site based on previously conducted optimization studies. After preparation, the surfactant samples were chilled to 50° F and filtered to remove precipitated material that formed from contact with the amendments and the site waters.

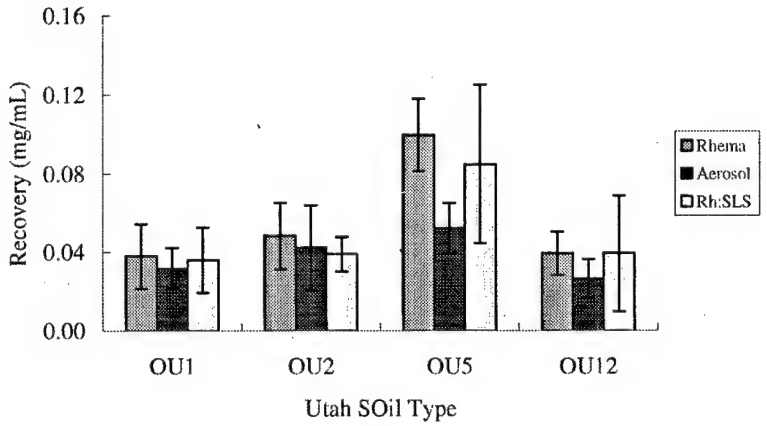
- Aerosol (2:1) ratio surfactant:isopropanol, 0.5% salt with all waters
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 3% salt in OU1, OU2, OU12
- Rhema Super Matrix (1:1) ratio surfactant:isopropanol, 4% salt in OU5
- Rhema/SLS (6:1) with (2:1) ratio surfactant:isopropanol, 3% salt in all waters

The results of the single extraction tests are summarized in Figure 4.2. The large standard deviations shown reflect slightly different methods used in collecting two sets of data. However, the trends shown reflect the important observations sets.

- In general, Rhema and Rhema/SLS performed better than aerosol,
- however, a detailed statistical analysis on this trend has not been performed and the relative differences between surfactants is not statistically important.

In all, single extraction tests recovered between 2 and 17% of the initial TCE spike.

Figure 4.2: TCE Recovery for Batch Extraction Combined data sets, Methods 1 and 2



Hydraulic conductivity analysis of the soils packed in columns was performed. These data are an indication of whether a soil is amenable to surfactant flooding or not. The soil conductivity for OU1 and OU 12 were moderate and represent soils that are well suited for surfactant flushing technology, being between 2.0×10^{-3} and 2.7×10^{-3} cm sec^{-1}. Likewise, OU2 is a good candidate soil for *in situ* remediation with a hydraulic conductivity of 1.2×10^{-2} cm/sec. The OU5 soil conductivity is marginal, being 5×10^{-4} cm/sec, but still within acceptable ranges of conductivity.

- OU1 hydraulic conductivity = 2.0×10^{-3} cm sec^{-1}
- OU2 hydraulic conductivity = 1.2×10^{-2} cm sec^{-1}
- OU5 hydraulic conductivity = 5×10^{-4} cm/sec^{-1}
- OU12 hydraulic conductivity = 2.7×10^{-3} cm sec^{-1}

Column extractions of TCE from various TCE-spiked Utah soils (labeled OU1-OU12) were performed using different surfactant mixtures in Utah waters. The surfactants were tested by preparation in the site waters. The mixtures with salt and co-solvent were the same as shown for the shake vials.

- Rhema and Rhema:SLS surfactant mixtures visibly contained precipitates at the extraction temperature of 50° F resulting in flow problems. As a result, out of eight column extractions using Rhema and Rhema:SLS mixtures, only two resulted in complete surfactant flow through the soil columns.
- Aerosol easily flowed through the soil columns with almost 100 % surfactant recovery.
- The amount of TCE extracted appears to be independent of the amount of surfactant that passed through the soil column. Overall, 0.08 – 2.2 % TCE was recovered in 3 out of 12 extractions. In all other tests, the amount of TCE extracted was not detectable.
- To minimize volatilization of the TCE before sample extraction, a second set of soil samples were spiked and packed in a TCE-saturated atmosphere. In addition, all surfactant solutions that had precipitates were filtered to improve flow through the soil columns. Trends in flow patterns and TCE removal were not appreciably different than for the first set.

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APPENDIX A

List of Symbols, Abbreviations and Acronyms

Acronym	Name
AFB	Air Force Base
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
DCA	Dichloroacetate
DCE	Dichloroethylene
DNAPL	Dense non-aqueous phase liquid
DoD	US Department of Defense
EPA	US Environmental Protection Agency
EPRI	Electrical Power Research Institute
ESTCP	Environmental Security Technology Certification Program
FFA	Federal Facility Agreement
FID	Flame ionization detector
GC	Gas chromatography
GWRTAC	Ground-water Remediation Technologies Analysis Center
HS	Sealed headspace
HSGC	Headspace gas chromatography
LNAPL	Light non-aqueous phase liquid
MBI	MBI International
NAPL	Non-aqueous phase liquid
ORP	Oxidation reduction potential
OU	Operating Unit
PAH	Polycrylic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCE	Perchloroethylene
RCRA	Resource Conservation and Recovery Act
SEAR	Surfactant Enhanced Aquifer Remediation
SERDP	Strategic Environmental Research and Development Program
SLS	Sodium lauryl sulfate
SOW	Statement of Work
SVE	Soil vapor extraction
TCA	1,1,1-trichloroethane
TCE	Trichloroethylene
TPH	Total petroleum hydrocarbons
UDEQ	Utah State Department of Environmental Quality
USU	Utah State University
VOC	Volatile organic compound

APPENDIX B

The RETEC Group, Inc. Final Report

Final Report for Preparation and Optimization of TCE Degrading Granular Cultures

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December 10, 2002

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December 10, 2002

1. Introduction and Background

MBI International (MBI) has developed a proprietary anaerobic granular mixed culture that has the capability to completely transform (dechlorinate) TCE and other chlorinated ethenes to non-chlorinated end products. The potential for using these granules for bioaugmentation of soils and groundwater, contaminated with TCE and cis-DCE (cDCE), at Hill AFB was the focus of an DoD funded project conducted cooperatively by MBI and Utah State University.

The objectives of this work effort, subcontracted to The RETEC Group, Inc. (RETEC), were to grow a sufficient mass of the granular TCE-dechlorinating culture for use in microcosm and soil column testing performed by Utah State University and to determine the costs of growing the culture at the scale needed if bioaugmentation were to be conducted at field scale. Testing of the kinetic transformation capacity of the granules was also undertaken to determine the maximum rates of transformation possible with this culture and allow a basis of comparison of rates achieved during the microcosm testing. RETEC also supplied Utah State University with a dispersed cell culture capable of dechlorinating PCE and other chlorinated ethenes referred to as the "Bachman Road" culture for comparative testing. This culture has been successfully used for bioaugmentation at two sites.

2. Materials and Methods

2.1 Bioreactor Operation

Approximately two liters (settled volume) of granules were obtained from MBI and cultured under anaerobic conditions in a seven-liter working volume, 15-liter total volume columnar, Upflow Anaerobic Sludge Blanket (UASB) reactor. This reactor, shown schematically in Figure 1, was constructed using glass, Teflon and stainless steel in all contact areas.

The design operating conditions were selected based on previous work conducted with these granules (Wu personal communication March 2002). Conditions are shown in Table 1.

Table 1. Summary of Reactor Design Operating Conditions

Parameter	Units	Value
Organic Loading Rate	kg COD/m^3-d	12.0
Inlet COD Concentration	mg/L	1240
TCE Loading Rate	g TCE/m^3-d	4.9
Inlet TCE Concentration	ug/L	500
System HRT	hrs	10

Chemicals:

Lactate was used as the sole added electron donor and carbon source. Sodium lactate (60% solution) was obtained from J. M. Swank Co. (Chicago, IL). This was diluted to a 7.5% solution for metering into the reactor.

Nutrients were added continuously based on a COD/N/P/S ratio of 100/1/0.2/0.2. Nutrients were made up in 44 gallon batches using the following: urea 5.2 g, ammonium sulfate 0.96 g, monobasic ammonium phosphate 2.16 g, magnesium chloride 1.36 g, calcium chloride 0.88 g, potassium chloride 0.44 g, and sodium chloride 0.59 g.

In addition a trace mineral solution was pulsed into the reactor on a two times per week basis to ensure sufficient concentrations of these metals were available. The metal mixture included molybdate, boron, nickel, manganese, zinc, copper, cobalt and iron.

The feed water for the reactor was industrial grade water that was nitrogen purged for removal of any dissolved oxygen. The reactor was operated at ambient temperature, which varied from 16° to 20° C during the course of the study.

2.2 Analytical Methods

Analysis of the effluent for COD and VOC were conducted on a 2-3 times per week schedule. System flow rate and pH were measured daily.

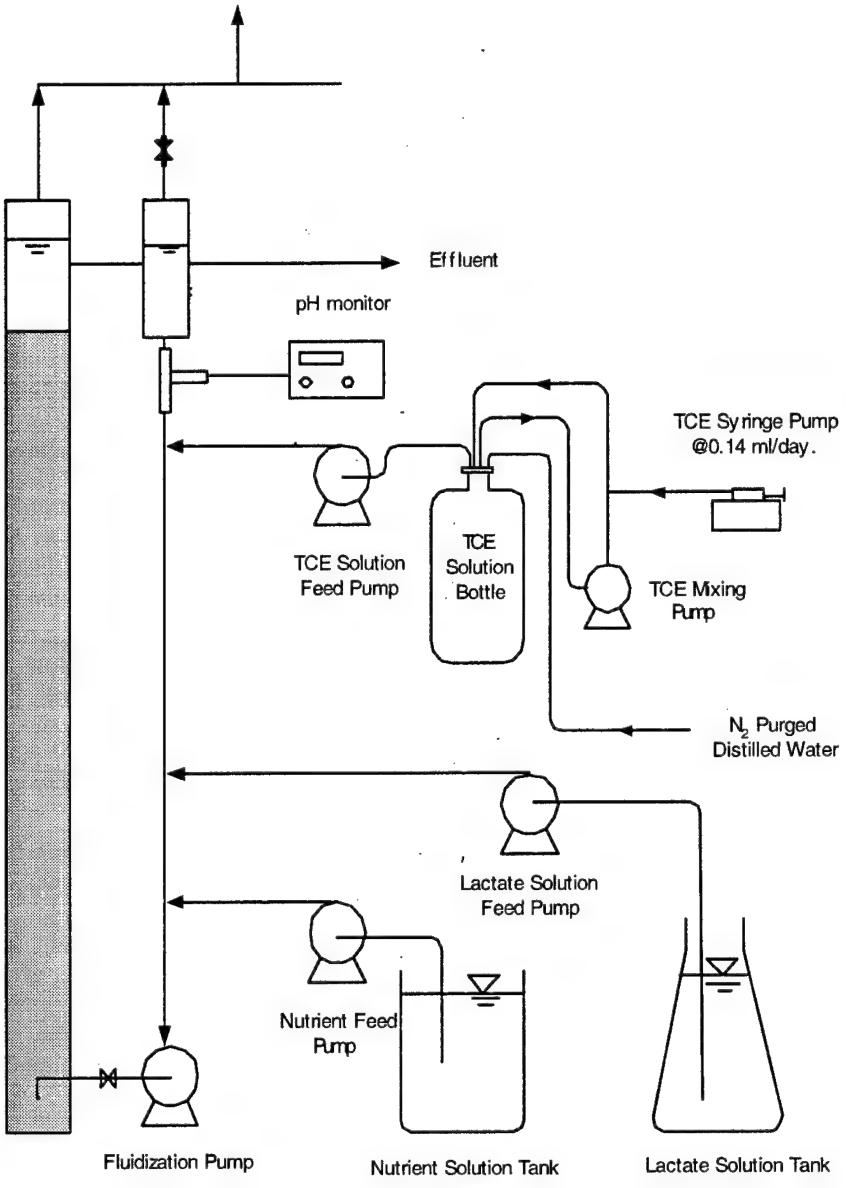


Figure 1. Schematic of UASB Reactor Used to Grow TCE Dechlorinating Granular

Total suspend solids (TSS) were determined using Standard Method 209C, (APHA, 1995).

COD analysis was performed in accordance with Standard Method 5220D (APHA 1995).

The pH was determined using an Accumet portable pH meter (Fisher Scientific) equipped with a Thermo Orion sure-flow combination pH probe.

VOC were analyzed using gas chromatography with a flame ionization detector (GC-FID) and static headspace method. Briefly this method is as follows. Duplicate samples (10 mL) were withdrawn from the reactor using a 20mL glass syringe and gently dispensed into a 22mL headspace vial using a 22-gauge needle. The headspace vials contained two drops of concentrated phosphoric acid and 2 grams of NaCl for enhanced recovery (salting out) of the TCE, cDCE and VC during static headspace GC-FID analysis. The vials were immediately sealed with Teflon coated septa and aluminum crimps. Samples were logged on the sample log form. Samples were usually analyzed on the same day. If not, they were stored at 4°C for a total of 72 hours or less before analysis. Samples prepared this way were placed into a Hewlett Packard Model 7694 headspace sampler connected to a Hewlett Packard 6890 series GC-FID. Separation was accomplished using a Supelco VOCOL column (105 meter length, 530 um ID, 3.0 um film thickness). Detection was with a flame ionization detector. GC conditions are presented in Table 2.

Table 2. GC conditions for chlorinated compounds analysis

Injector	septum-equipped programmable on-column Injector
Column	105 m VOCOL capillary column (Supelco), 0.53 mm I.D. 3 µm film
Detector	a capillary FID, with a ceramic tip
Injector Temperature	220°C
Detector Temperature	230°C
Autosampler:	a HP 7694 Headspace sampler with 44-sample carrousel

External calibration procedures were used for quantification. Five to seven level calibration curves were prepared from stock solutions in methanol. Calibration curves were generated for individual components, with concentrations ranging from 1 ug/L to 1000 ug/L. A linear regression, with forced origin, was used to calculate the calibration factors. Check standards were run with every sample batch and curves updated if the response varied by more than 10% from the previous response. Retention times were updated with each standard injection. Reagent blanks were run with every batch of samples to verify no sample contamination or carryover occurred from the headspace apparatus.

2.3 TCE Transformation Rate Experiment

The experiment conducted to determine the dechlorination rates of the granular culture were performed using granules taken directly from the reactor using a 100 ml Perfektum glass syringe.

Serum bottles (160 mL) containing a total mixture of 85.2 mL of: 0.2 ml of resazurin (0.1 %), 2 ml of basal salt, 0.5 ml of Na_2S solution, 2 ml of buffer, 0.5 ml of lactate (60 %) and 80 ml of oxygen-free tap water, added under strict anoxic conditions Shelton and Tiedje (1984). The serum bottles were capped using Teflon septum with mini-inert syringe valves on the top. The headspace of the bottles were evacuated and replaced with N_2 three times, pressurized to approximately 1.3 ATM, and then amended with the inoculum. Pure TCE (0.5 uL) was added via syringe to achieve an initial concentration of approximately 18 uM. All of the bottles were placed on a speed-controlled shaker operated at room temperature. Samples were prepared in quadruplicate for active and triplicate for controls.

The serum bottles were sampled periodically over a four day period. For each sample, two (2.0) ml of aqueous sample was taken from the serum bottles and transferred to headspace vials containing 8 mL of distilled water, acid preservative and NaCl, as indicated previously to enhance recovery of the VOC. The headspace vials were then sealed with Teflon lined septa and aluminum crimps. The samples were either immediately analyzed or stored at 4°C until analyzed. All samples were analyzed within 72 hours.

Chemicals:

Sodium Lactate (60%) was U.S.P. grade, manufactured by Ferro Pfanstiehl Laboratories, Inc., distributed by J.M. Swank Co.

TCE was HPLC grade, obtained from Sigma-Aldrich Co. All other chemicals used were reagent grade obtained from either Fisher Scientific or Sigma.

The basal salts medium consisted following per liter; NaCl, 50 g; $MgCl_2$ $6H_2O$, 25 g; KH_2PO_4, 10 g; NH_4Cl, 15 g; KCl, 15 g; $CaCl_2$ $2H_2O$, 0.75 g.

The trace element solution has following chemical per liter; HCl, 10 ml; $FeCl_2$ $4H_2O$, 1500 mg; $CoCl_2$ H_2O, 190 mg; $MnCl_2$ $4H_2O$, 100 mg; $ZnCl_2$, 70 mg; H_3BO_3, 6 mg; Na_2MnO_4 $2H_2O$, 36 mg; $NiCl_2$ $6H_2O$, 24 mg; $CuCl_2$ $2H_2O$, 2 mg.

The buffer (pH 6.5) was a combination of 56.5 ml of NaH_2PO_4 H_2O (0.2 M) and 43.5 ml Na_2HPO_4 $12H_2O$ (0.2 M) added to 200 ml of distilled water.

The Na_2S solution had 48 g of Na_2S $9H_2O$ in one liter of distilled water.

3. Results

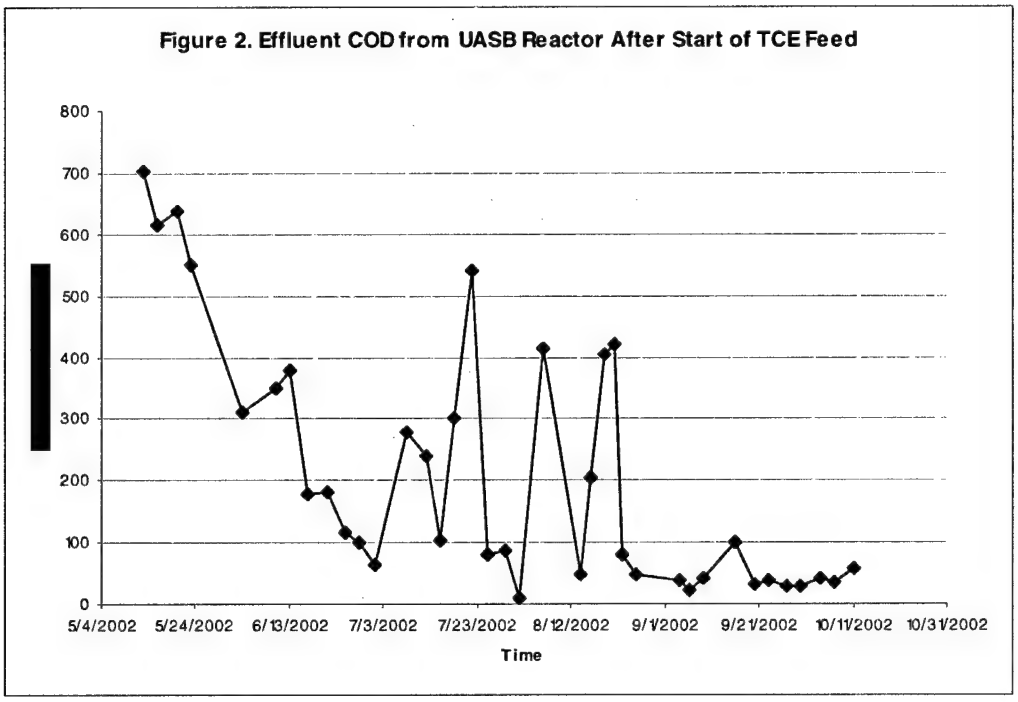
3.1 Reactor operation

The reactor was started on March 13, 2002 using approximately two liters of granules that had been stored at room temperature for over a year. When the inoculum was added to the reactor it was observed that although some granules remained intact, a considerable amount had disintegrated into small floc particles (most of the granules had disaggregated during storage). Because of this observation, it was decided to initially feed the system just substrate (without TCE) and try and obtain a granular culture. The influent flow rate was set to approximately 83 mL/min. This resulted in a system HRT of approximately 3 hours (based on overall volume of 15 Liters). This was done to help washout the small floc particles. The system was initially operated at an applied organic loading rate (OLR) of 4 kg COD/m^3-d. The nominal inlet COD based on this OLR and hydraulic throughput was approximately 125 mg/L. This was done to prevent accumulation of high concentrations of volatile fatty acids while the culture was recovering. The reactor was fed lactate as the sole organic substrate in nitrogen-purged water; macronutrients were added separately via a metering pump. Micronutrients were pulsed to the reactor twice weekly. The pH was adjusted to around 7.0 using a sodium bicarbonate solution.

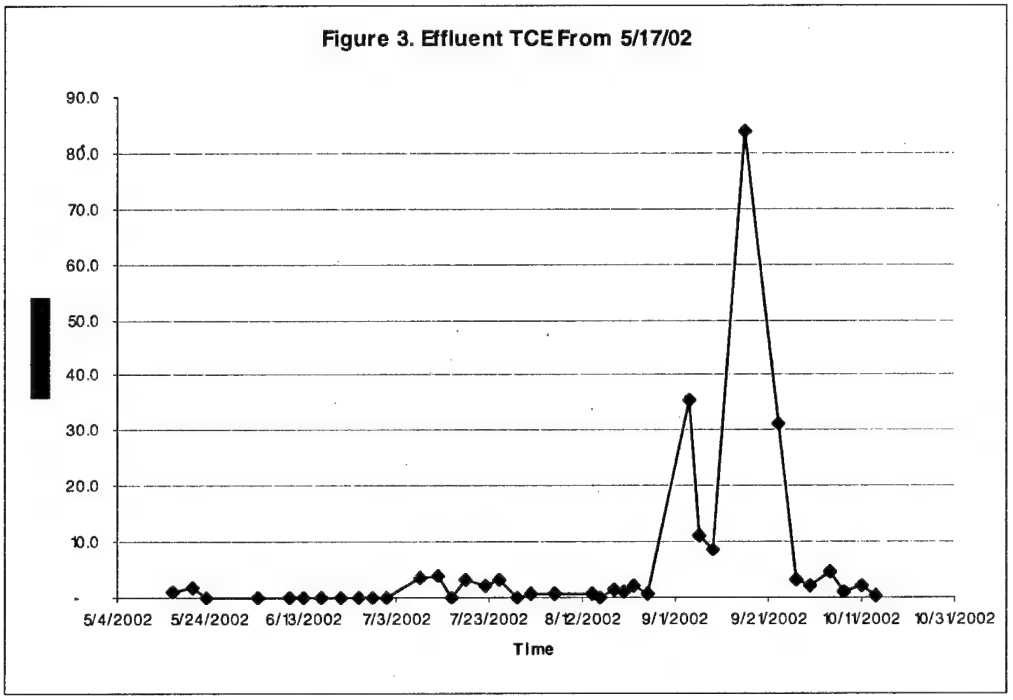
Once gas production was visually observed, the lactate feed was increased in steps, ultimately to achieve the design OLR of 12 kg COD/m3-d by April 4th. The feed flow rate was then decreased to 55 mL/min (April 9) and 25 mL/min (April 24) as the flocs were washed out of the system.

On May 13th TCE addition was started at 0.5 mg/L; the resulting applied TCE loading rate was 4.9 mg/L-d. Monitoring of the system on a 2-3 time per basis commenced for effluent TCE and COD.

Results, starting from the first sampling date (May 16) through October 14, are presented in Figures 2 and 3 for COD and TCE, respectively. As can be seen, initially effluent COD was quite high (approximately 700 mg/L) after addition of the TCE was initiated. Based on the rate of lactate addition the feed COD concentration would have been approximately 1240 mg/L so removal was close to 45% at this time. Since COD was not measured prior to this time it is unclear whether there was a cause and effect or if the COD removal efficiency was the same prior to adding the TCE. Removal efficiency increased steadily with effluent COD decreasing to below 100 mg/L by June 28th. Several times during July and August it was observed that the effluent COD increased to between 400 and 500 mg/L. In each instance this could be attributed to a pH excursion out of the range suitable for acetate catabolizing methanogens (6.6 to 7.4). Once the system pH was brought back into this acceptable range, effluent COD concentrations rapidly returned to pre-pertubation levels.



Removal of TCE was high from the onset and remained that way with the exception of a couple of excursions observed during September. The reason(s) for these somewhat anomalous results are not known. There were no pH excursions or other factors that could be related to the observations. The performance of the system stabilized by September 23rd; effluent TCE remained below 5 ug/L for the remainder of the project.



The reactor was shut down on October 22nd. Four liters of granules were shipped to Utah State University (USU) and one liter kept in case there is a need to restart the system. Three liters of granules were shipped previously to USU during late July.

3.2 Batch TCE Transformation Rate Tests

The results of the dechlorination activity testing are summarized in Figure 4, the average of 4 replicates; error bars represent the standard deviation. Dechlorination followed the typical pattern of sequential transformation with TCE being close to stoichiometrically converted to cis-DCE (cDCE) and to vinyl chloride and then to ethane (not quantitated). No conversion of TCE was observed in the abiotic controls.

The concentration of TCE declined rapidly (from 19.1 uM to 5.9 uM) over the course of the first day and then more slowly over the next two days to non-detect levels. The concentration of cDCE increased proportionally to the decrease in TCE. The concentration of cDCE concentration was 13.4 uM by the end of the first day. The concentration of cDCE remained approximately constant until TCE was depleted and then decreased precipitously over the following day.

The concentration of VC gradually increased over the first 3 days, peaking at 4.9 uM. The VC concentration decreased to 2.1 uM over the next 8 hours, once the concentration of cDCE declined to low levels.

Figure 4. TCE Degradation Kinetic

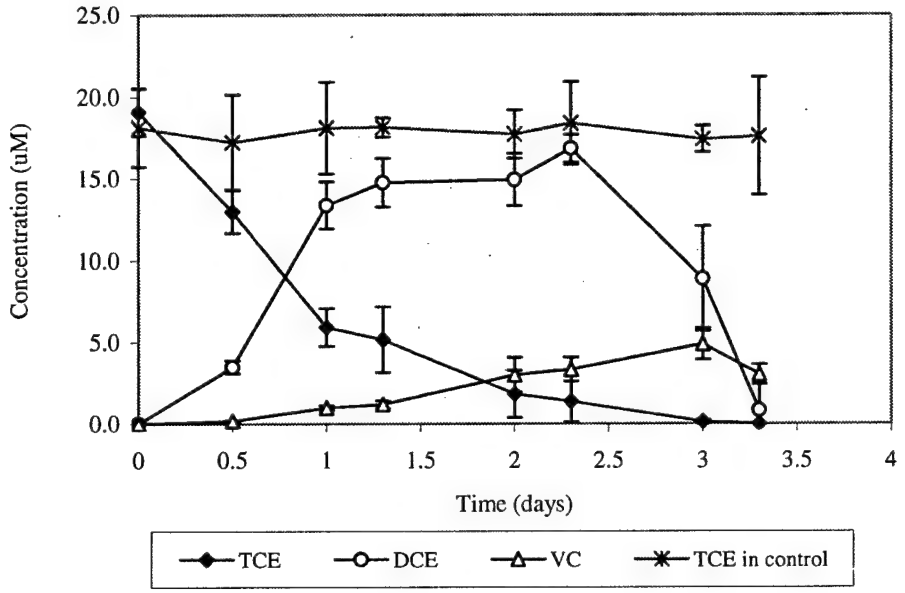
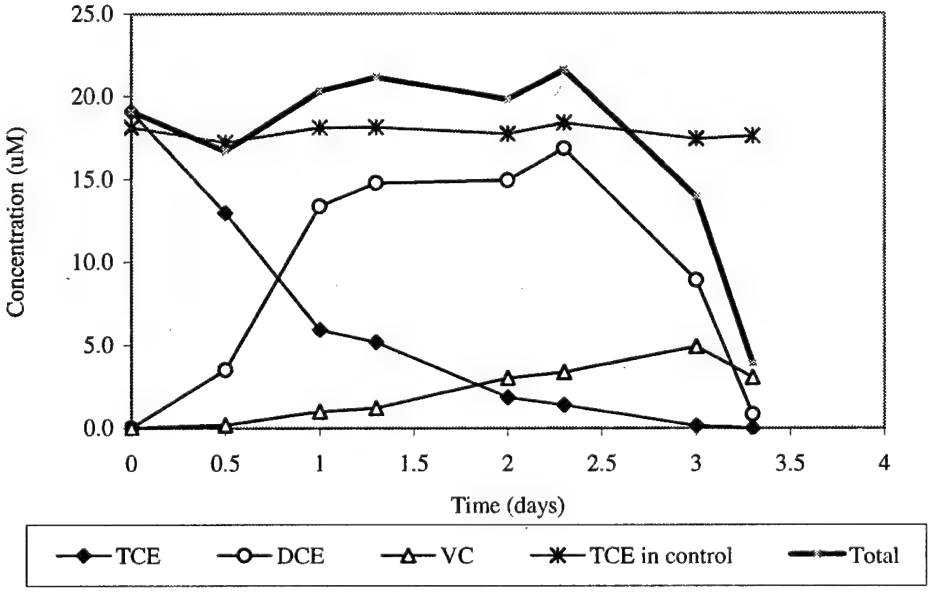


Figure 5. Total Mass of Chlorination Compounds

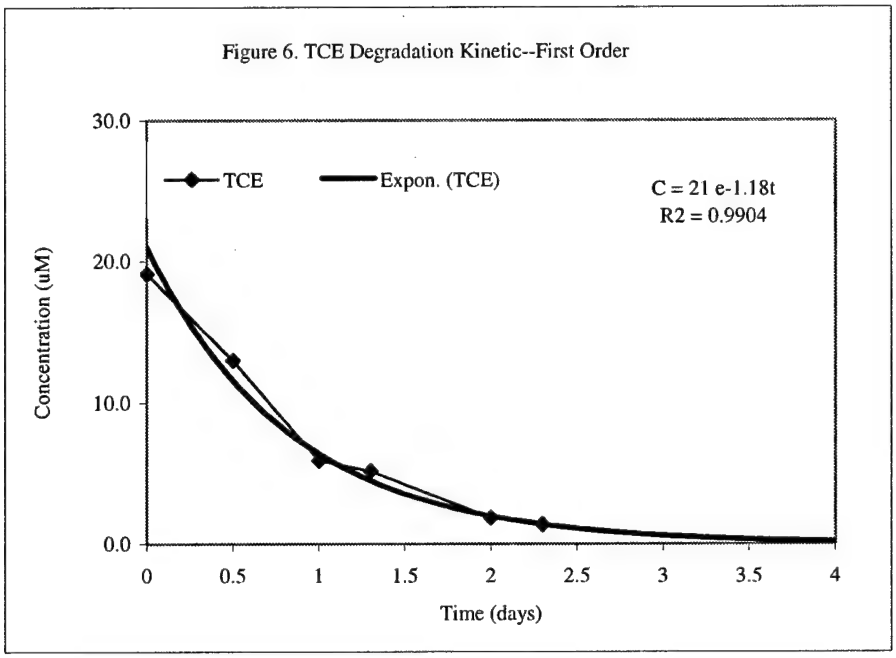


The results of the total mass of the chlorinated ethenes on a molar basis are shown in the Figure 5. The total mass in the aqueous phase remained essentially constant until TCE was reduced to low levels and then decreased sharply thereafter as cDCE was reduced to VC and then ethane.

In the control samples, containing medium, trace nutrients and nitrogen purged tap water only (no inoculum), the TCE concentration reminded at an average concentration of 17.7 uM. No loss of TCE was observed.

The degradation curve for TCE was fitted to first-order reaction kinetics with k value of 1.18 day^{-1}. The average TSS concentration was measured to be 4.72 g/L. Results are presented in the Figure 6. There was insufficient data upon which to calculate kinetic rates for transformation of cDCE and VC.

Figure 6. TCE Degradation Kinetic--First Order



4. Feasibility Design for Scale up to Field Application

The site at Hill AFB, where bioaugmentation is being considered for field-testing, is a shallow site with poorly graded silty sands. Depth to water varies from 20 to 50 feet. There is a saturated thickness of 30 to 50 feet above a tight confining layer. The width of the TCE plume varies from 300 to 500 feet; total plume length is approximately 5000 feet.

To obtain a preliminary estimate of the volume of granules needed, a remediation strategy had to be selected. It was decided that an in-situ bio-barrier was the most practical approach for this site. In order to effectively do this with the MBI granular culture, it would be necessary to disrupt the granules sufficiently so they could be added to the subsurface without causing plugging or significant reduction in the hydraulic conductivity. Potential methods to achieve this were not considered in this feasibility work. It was assumed that a method could be developed such that disrupted granules could be delivered over a volume sufficient such that there would be enough reaction time to convert all the TCE to non-chlorinated end products. For the purposes of this feasibility exercise, the width and depth of this area was set to the average values of 400 feet and 40 feet respectively. Results from preliminary microcosm testing conducted at Utah State University (USU) were used to estimate the length of the "reaction zone" needed.

Results from microcosm testing at USU indicated that 21 days were needed to convert all of the TCE to cDCE; another 21 days were observed required to convert the cDCE through VC to ethene as the primary end product. This was accomplished with a 1% (v/v) inoculum. A total of 42 days treatment time in the bio-barrier was, therefore, assumed for the purposes of this feasibility assessment. Average groundwater velocity at the Hill AFB site is approximately 0.4 feet per day. The bio-barrier would, therefore, have to be approximately 17 feet in length to achieve complete transformation of the TCE to non-chlorinated end products. The total volume of the treatment zone or bio-barrier would then be:

40 feet x 400 feet x 17 feet = 272,000 cubic feet.

The pore volume of the soils at Hill AFB average approximately 30%. Using this to calculate the liquid volume of the reaction zone and assuming an inoculum of 1% is needed, the total volume of inoculum comes to 6,100 gallons.

Different options for production and delivery of 6,100 gallons of granular inoculum were screened. These included construction of a large UASB reactor at the site, construction of a system at MBI (and subsequent transport of granules to the site) and production of "seed" inoculum at MBI followed by transport of the harvested granules to the site to start several small, skid-mounted units. The latter option was pursued for this analysis because of the lower costs, flexibility, leverage of existing equipment at MBI/RETEC and ability to subsequently reuse the skid-mounted unit(s)

for other sites. Skid mounted units can be housed in temporary, relatively small buildings and major costs associated with siting, foundations, etc. avoided. In short, commissioning, decommissioning and demobilization costs are small compared to construction of a large system at either Hill AFB or MBI, and logistics are simplified.

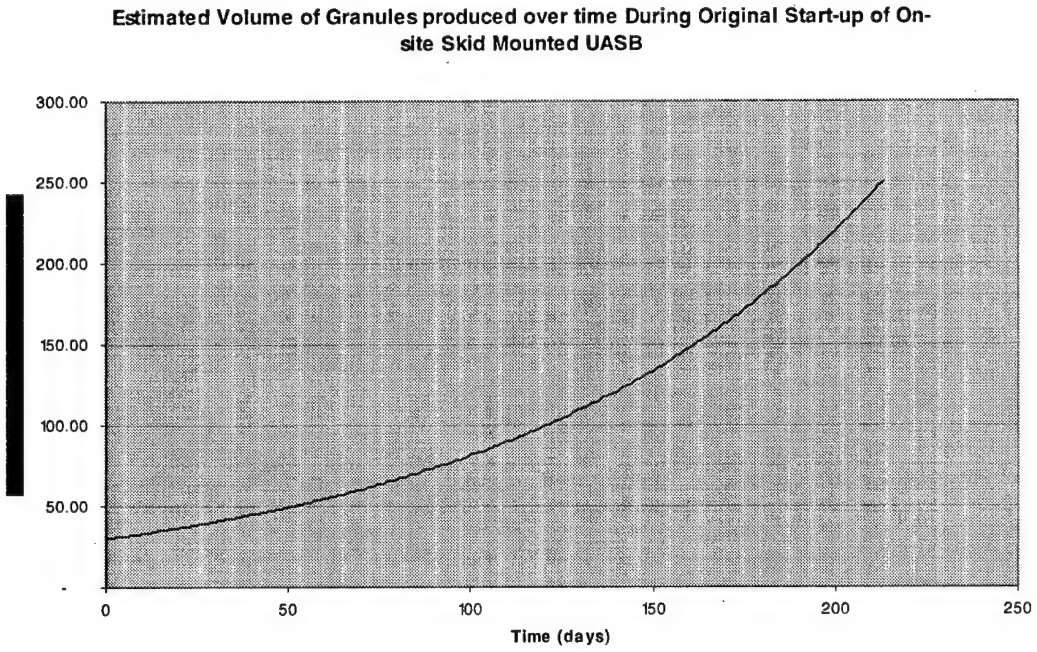
There is a breakpoint in size where the economic advantage of using skid-mounting equipment, versus field erection of systems, becomes poor. For systems such as UASBs and Fluidized Bed Reactors (FBRs) this occurs when reactors are about 6 foot in diameter. This is generally due to the size of the piping and ancillary equipment required and, therefore, size of the skid needed. For this exercise the maximum reactor diameter was set to 6 feet.

Based on this size, an inoculum volume required for one-quarter of the plume width (10 feet) can be produced at a time. RETEC has created in-situ bio-barriers of this "zone of influence" successfully in two other field projects for bioaugmentation of halorespiring anaerobic bacteria. The plan would, therefore, be to sequentially grow and inoculate the site in four separate events. The use of two skid-mounted reactors was selected to allow completion of the project in a reasonable time period.

The maximum volume of granules that RETEC can supply to MBI using existing equipment is approximately 210 gallons (30 cf). This represents approximately a 12% inoculum for starting the six-foot diameter skid-mounted UASB reactor systems. Based on maintaining an applied organic loading rate of 12 kg COD/m-3-d and a 5.0% yield (mg biomass per mg of COD), the estimated time to produce 250 cf of granules would be approximately 210 days Figure 7. Eighty percent of this volume is sufficient for one inoculation event. The remaining 20% would then be used to grow the second batch of inoculum (200 cf) over an additional 140 days. The second UASB would be operated off set in time to the first system by approximately 70 days. A second batch of starter culture for the system could be produced within this 70-day time frame. This would allow all the inoculation to be conducted over a 210 day period with inoculation events every 70 days starting at a time 210 days after the initial UASB is started at the site. Total time from start-up of the first system at Hill AFB until shutdown would be 420 days, or roughly 14 months. Allowing several months for site preparation and equipment start-up and an additional several months for decommissioning and demobilization, the overall time on-site would be approximately 18 months. Although this is somewhat protracted the timing is not necessarily prohibitively long. It does, however, significantly add to the operational expense associated with manpower costs.

The cost of the skid-mounted UASB reactors was estimated based on direct experience with designing and constructing this size FBR and conversations with equipment vendors. The cost to design and construct such a system would be $280,000 to $300,000. Picking the mid-point, the cost would be approximately $580,000 for two units.

The cost of chemicals, including sodium lactate, pH control, nitrogen (urea), phosphorous and trace minerals was estimated to be $3,100. This was based on using a COD/N/P/S ratio of 100/1/0.2/0.2. Addition of trace minerals was assumed based on achieving a set concentration in the reactors via pulse addition twice per week. The major cost element was sodium lactate estimated at $2,625 for 3000 pounds of 60 % sodium lactate.



The major operating costs are for manpower to operate and monitor the system and analytical needed for process control. Using 14 months of on-site operation, of a full-time operator at $40/hr the cost of operations would be just over $97,000 per year. Add to this an additional $25,000 for supervisory support and technical oversight, the total manpower would be $122,000. This cost excludes any travel costs and manpower costs associated with shipping, installation, and start-up.

Analytical was assumed limited to COD (performed three times per weeks on-site) and VOC (performed three times per week in a contract lab). A total of 61 weeks was assumed. At $70/sample for VOC and COD estimated to cost $10/sample (for supplies and disposal of residuals) the total cost of analytical comes to approximately $14,640.

A summary of the estimated operating costs is shown in Table 3 below. Note, for the purposes of this exercise the costs associated with power for pumps and system heating were not included. These cost are believed to total under $15,000 for the 14 months of operation. The capital cost of the heating system was included in the cost of the skid-mounted units.

Item	Total Estimate Cost
Manpower	122,000
Analytical	14,640
Chemicals	3,100
Total	139,740

Also excluded from this analysis were the method and associated costs for disrupting the granules into a slurry form that can be delivered into the subsurface and all the costs of design, installation and operation of the series of well and above ground equipment (e.g. feed tanks, pumps, mixers, etc.) to form and the bio-barrier and maintain its viability and dechlorinating capability.

We have not done scale-up on the Bachman Road culture past the 300-gallon reactor used to date. So we do not have reliable cost figures. Based on being able to successfully concentrate the culture using membranes ten-fold (testing is underway), the cost of production in the existing system located at MBI is estimated to be approximately $75,000. This figure does not include transportation and associated costs. The ability to achieve the projected level of cell concentration of the culture is still being investigated.

5. Conclusions

Based on results obtained during this work effort and microcosm testing conducted at Utah State University, the MBI granular culture is capable of completely dechlorinating TCE and its daughter partial dechlorination products.

A feasibility assessment was conducted on the cost of growing sufficient inoculum to treat a target plume at Hill AFB. It was decided that production could best be accomplished using two skid mounted UASB reactors that could be located at the site, thereby eliminating logistics and costs of transporting large volume of anaerobic inoculum long distances or the excessive costs of designing and installing a field erected system at the site. The potential for reuse of the systems at future additional sites and avoidance of major site preparation, permitting and other costs associated with a large single system located either at MBI or the site were additional reasons for the selection of this approach. The cost of the equipment skids was estimated to be $580,000. This is exclusive of costs for a temporary structure to house the units and cost of shipment and installation, start-up decommissioning and demobilization.

The estimated cost of operation was determined to be approximately $140,000. This figure is inclusive of manpower, analytical and chemicals. It does not include costs for design, installation and operation of the pumps, wells and other ancillary equipment needed to deliver the inoculum to the subsurface and operate the bio-barrier. Costs and methods needed to physically disrupt the granules, so they could be injected into the subsurface to the form the bio-barrier, were also beyond the scope of this exercise. Note these are not trivial and development of a suitable technique will require additional R&D efforts.

Further work on soil column testing (to be conducted at USU) followed by a considerably more detailed cost analysis is required to better define the true economic feasibility of this approach. Several technical hurdles, primarily development of a method to sufficiently disrupt the granular culture, while not adversely affecting its viability to dechlorinate TCE, so the culture can be effectively delivered to the subsurface to form the bio-barrier while retaining good hydraulic conductivity, need to be overcome for this to become a practical option.

Timing is a major issue. The growth of the biocatalyst is the rate-limiting step in the process and this is a fundamental constraint due to the slow growth rate and yield of the consortia that form the granules. An effort was made to lessen the impact of this by "staging" the inoculation in four events. The total time was estimated to be 18 months from set-up of the skid-mounted UASB's on-site until demobilization. This does not include the time and costs associated with production of sufficient granular culture to start-up the reactors at the site or time required to design and construct the skid-mounted units.

These limitations and constraints not withstanding, this is still a potential option for a site such as Hill AFB where it appears that biostimulation is not effective and bioaugmentation will be required to effect in-situ treatment.

APPENDIX C

Utah State University Final Report

Evaluation of the Effectiveness of Microbial Innocula Amendment to Stimulate TCE Dechlorination at OU5, Hill AFB

Submitted to

MBI, Inc.
Lansing Michigan

Submitted by

Utah Water Research Laboratory
Utah State University
Logan, Utah

March 24, 2003

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Evaluation of the Effectiveness of Microbial Innocula Amendment to Stimulate TCE Dechlorination at OU5, Hill AFB

1. INTRODUCTION

This report describes activities performed in response to BAA-TYN-01-001, issued by Tyndall AFB, 01 December, 2000, that comprise the first year of a 3-year laboratory to pilot to field demonstration treatability study to evaluate an innovative biological treatment method for the remediation of chlorinated hydrocarbon-impacted soil and groundwater, common at facilities throughout the Air Force system, at a TCE contaminated site, OU5, at Hill AFB, UT. The report details the design of the experiments, and the collection and analysis of data generated to assess the effectiveness of the augmentation of a microbial innocula in stimulating the complete dechlorination, of TCE in simulated aquifer systems constructed of soil and groundwater from this contaminated Hill AFB site. The innocula used in the study were provided to USU by MBI. This study was conducted as a complement to an on-going Hill AFB-funded project to assess carbon donor addition alone as a means of stimulating dechlorination in indigenous microbial populations. Results of the carbon donor study have also been incorporated into this report so that the response of the aquifer system to microbial amendment could be fully demonstrated relative to carbon donor stimulated systems used as active controls.

2. SITE BACKGROUND

2.1 Hill AFB History

Hill AFB is located in northern Utah, approximately 25 miles north of Salt Lake City, 5 miles south of Ogden, and adjacent to U.S. Interstate 5. The base occupies approximately 6,700 acres in Davis and Weber counties. The base is bounded on the west by the Davis-Weber Canal and Interstate 15, on the south by State Route 193, on the north by the Davis-Weber Canal, and on the east by private property.

Hill AFB and earlier military installations that occupied the Base have operated industrial facilities related to military aircraft since the construction of the Ogden Air Depot in 1940. Many different aircraft, missile, and weapons systems have been maintained and tested in the course of continuous operation and expansion of the Base since its inception. These operations and past practices for waste disposal have resulted in the contamination of the soils and groundwater at the Base. This contamination has also migrated off-Base via the groundwater migration pathway.

Hill Air Force Base was put on the National Priorities List in 1987. According to the Federal Facility Agreement (FFA) between Hill AFB, EPA, and the State of Utah Department of Environmental Quality (UDEQ), Hill AFB is committed to completion of

ongoing CERCLA activities and is actively seeking innovative technologies to reduce the cost of required remediation efforts across the base.

Hill AFB overlies three aquifers of which two are productive drinking water sources and are used by Hill AFB and the surrounding communities. In addition, there are seeps and springs, and field drains along the hillside south and north of the Base that intercept historical waste petroleum and cleaning solvent disposal areas containing BTEX, PCE, TCE and other contaminants at numerous discharge points.

The innovative microbial augmentation technology investigated in this study are relevant to a number of sites that exist throughout HAFB. Free product pools and residually contaminated soil exist within areas of OU1 and OU2, and significant off-site, dissolved chlorinated solvent plumes are associated with OU1, OU2, OU4, OU5, OU6, and OU9. The nature of soil and groundwater contamination found throughout Hill is not uncommon in the Air Force system, and this bioagmentation technology has the potential to provide significantly lower cost options for plume containment and control, source treatment, and overall site management necessary to reduce the risk of past disposal practices to the receptor community than many currently available options. Table 2.1 summarizes the nature of past practices and resultant contamination at a number of Hill sites where accelerated dechlorination provided by this bioaugmentation technology could be used to reduce the cost and increase the effectiveness of remediation activities.

Table 2.1. Hill AFB sites for which accelerated dechlorination bioaugmentation technology is applicable.

Site	History	Contaminants	Media
OU1	Landfills, Chemical Disposal Pits, Fire Training Area	LNAPL, Chlorinated Solvents	Free Product, Soil, Groundwater
OU2	Chemical Disposal Pit	DNAPL, Chlorinated Solvents	Free Product, Soil
OU4	Landfill, Road Side Dumping	Chlorinated Solvents	Soil, Groundwater
OU5	Open Waste Disposal, Leaking UST	Chlorinated Solvents, PCBs	Groundwater, Soil
OU6	Substation, Open Waste Disposal	PCBs, Chlorinated Solvents	Soil, Groundwater
OU9	Open Waste Disposal	Chlorinated Solvents	Groundwater

2.2 OU5 Remedial Actions

The following information was obtained from an OU5 site summary report prepared by Montgomery Watson (2000) in 2000.

As originally defined, OU 5 consisted of only the Toole Army Rail Shop (TARS) and Bamberger Pond sites. Initial investigations in these areas began as early as 1987, and Operable Unit 5 was carried through the CERCLA investigation process. The final RI (completed in 1995), Engineering Evaluation/Cost Analysis (EE/CA), and FS documents were prepared based on the understanding of the nature and extent of contamination that existed at that time. At the conclusion of the FS, two ground-water treatment systems were installed and are currently operating as interim remedial actions.

In 1998, an investigation of the OU 9 North Area concluded that a volatile organic compound (VOC) groundwater plume from a source upgradient of the TARS area was comingling with the TARS plume resulting in an affected area of groundwater contamination substantially larger than that addressed in the OU 5 RI. With the discovery of additional contamination, Hill AFB and regulatory agencies agreed to address the new contaminant plumes and source areas as part of OU 5. Consequently, the OU 5 RI was re-opened on in January 1999.

In an effort to reduce future impacts to off-Base receptors and minimize potential migration of contaminants, Hill AFB implemented two interim removal actions: a Phase I Aeration Curtain, and a Phase II Groundwater Extraction System (GES) (Figure 2.1). The aeration curtain was completed in June 1997 east of Main Street in Sunset, Utah, to prevent further degradation of groundwater quality by preventing the spread of contamination to unaffected residents living in Sunset and Clinton. The Phase II GES was installed in September 1997 approximately 400 ft west of the aeration curtain to provide capture of contaminated groundwater downgradient of the Phase I system.

The Aeration Curtain consists of an air sparge (AS) system and a soil vapor extraction (SVE) system, with an associated blower building. The AS and SVE systems are located in a 30-foot deep trench that extends 400 ft north to south. Ambient air is sparged into the contaminated aquifer, stripping off the VOCs in the groundwater. As the air travels upwards toward the ground surface, the SVE system captures the contaminated air and discharges it to the atmosphere via a stack. The system has operated continuously since June 1997, with the exception of several short-term shutdowns for maintenance activities. Throughout its history, the system has experienced operation problems with the sparge blowers due to excessive backpressure from the sparge lines. Despite these problems, the system has operated with a treatment efficiency in excess of 90 percent and an uptime in excess of 95 percent. However, overall TCE mass removal for the 3 years of operation summarized in the Montgomery Watson report totaled only slightly more than 5 pounds.

Of particular relevance to this study is the evidence of stimulated anaerobic dechlorination in response to the apparent release of degradable carbon to the aquifer from the guar biopolymer that was utilized during trench construction. Wells U5-132,

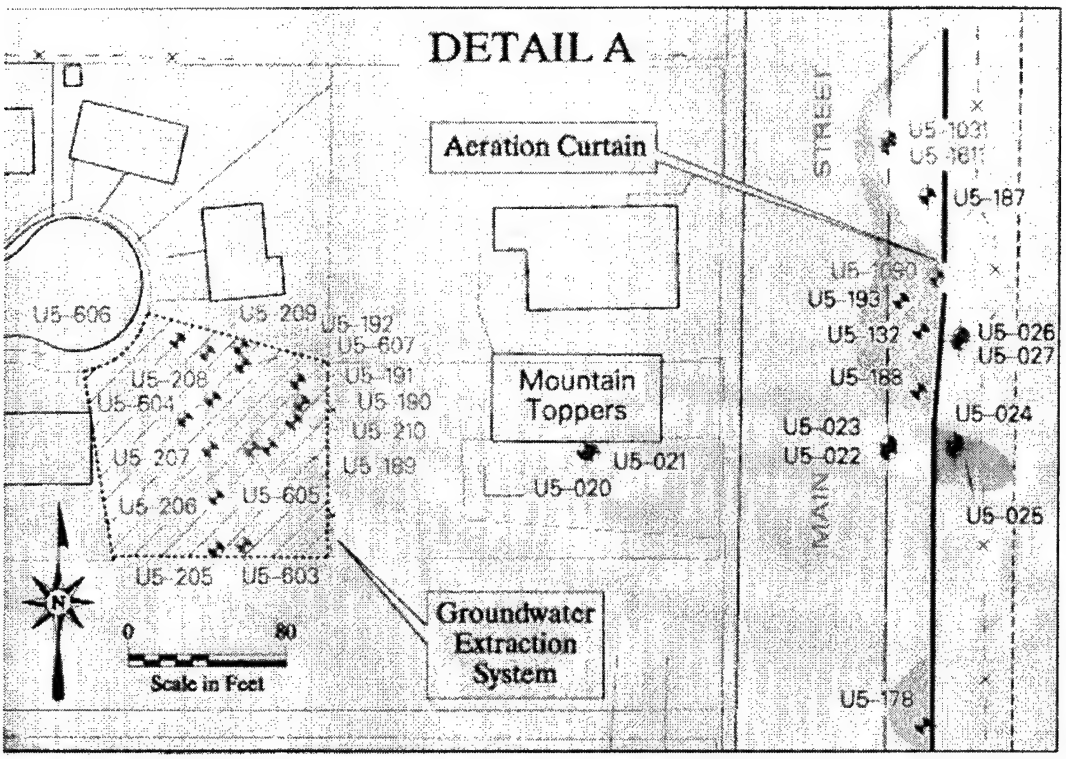


Figure 2.1. Details of air sparging and groundwater extraction remediation system implemented at OU5, Hill AFB, UT.

U5-188, U5-193, U5-020, U5-021, and U5-023 (Figure 2.1) all displayed some level of cis-DCE, and VC contamination that remained for 12 to 15 months following the installation and initiation of the aeration curtain in June 1997. Well U5-132 also showed evidence of dechlorination enhancement following a pilot air sparging test that was conducted near this well in late 1994 to mid-1995.

This observation of stimulated dechlorination with carbon donor addition lead Hill AFB, EM to conduct an evaluation of the OU5 groundwater plume in 2000. The focus of the evaluation was the feasibility of implementing enhanced biodegradation through carbon addition to the aquifer. The primary results of this evaluation were that the OU5 aquifer is carbon starved (i.e., insufficient mass of either naturally occurring or anthropogenic carbon) and thus incapable of significantly degrading TCE through reductive dechlorination. As part of the evaluation, an expert panel was convened in which individuals with experience in anaerobic reductive dechlorination and aquifer amendment with various carbon sources, including vegetable oil, gathered to discuss the remedial approach for the OU5 plume. The primary recommendation from that meeting was that Hill AFB should conduct a series of laboratory tests using various carbon substrates in order to gain additional information and insights into the the process before its full-scale implementation at Hill AFB.

Specifically the panel recommended that Hill should evaluate the effectiveness of stimulating :

"...anaerobic dechlorination ... using vegetable oil ... mixed with a solid in a trench. The panel does however (also) recommend that Hill consider alternative insoluble carbon sources at least in the early phases of laboratory testing. The panel recommends that Hill undertake two separate treatability-testing efforts. The first being a laboratory based flask/microcosm study to evaluate ... the presence of ...(the) anaerobic dechlorination process and to evaluate various other parameters such as carbon sources ... The second kind of treatablility testing the panel recommends Hill undertake is moderate scale column tests to evaluate effects such as partitioning of TCE into vegetable oil, methane production, noxious odor generation, and nutrient requirements. The panel does not recommend field scale treatability testing."

From these recommendations, laboratory studies were begun by the UWRL to investigate TCE dechlorination efficiency of OU5 aquifer solids in response to the addition of a variety of soluble and vegetable oil based carbon donors. Results from this carbon donor study failed to confirm the presence of TCE dechlorination capacity in the native OU5 aquifer solids, and lead to this study, evaluating the feasibility of enhancing the dechlorination capacity at OU5 with the addition of dechlorinating microbial consortia.

3. STUDY OBJECTIVES

The purpose of this study was to provide proof-of-concept and preliminary design data for the use of a microbial amendment to optimize chlorinated hydrocarbon-impacted groundwater remediation at OU5 at Hill AFB. The specific objectives of the study were to:

1. Verify that two microbial consortium, one a granular culture developed by MBI, and the other a suspended growth culture isolated from the Bachman Road site in Oscoda, MI, are capable of providing complete anaerobic dehalogenation of TCE in microcosms constructed from soil and groundwater collected from OU5 at Hill AFB, when these microcosms are amended with suitable carbon substrate
2. Evaluate the comparative effectiveness of various soluble and slow release carbon substrates to stimulate TCE dechlorination by these microbial consortium; and
3. Evaluate the effectiveness of zero valent iron to provide TCE dechlorination in this site groundwater for comparison with accelerated biological means for chlorinated hydrocarbon dechlorination.

These study objectives were met by the completion of laboratory batch microcosm studies using soil and groundwater collected from OU5 at Hill AFB, Utah. This Air Force facility was chosen for the collection of field samples because of its close

proximity to the Utah Water Research Laboratory, because of on-going UWRL projects at Hill that facilitate coordination and cooperation with Base EM personnel, and because its sites are representative of the range of problems that exist throughout the Air Force system.

4. EXPERIMENTAL DESIGN

This laboratory study consisted of a series of four sets of experiments designed to evaluate the effectiveness of microbial amendment in stimulating TCE dechlorination and to investigate abiotic ZVI-TCE reactions in OU5 aquifer material. The four experiments included: 1) a preliminary microbial innocula dilution study designed to determine the optimal mixed culture dilution required to produce dechlorination in spiked microcosms; 2) a preliminary range-finding study to determine rate of TCE transformation in response to a variety of carbon donor amendments, 3) a complete culture augmentation study to determine optimal carbon donor and microbial culture amendment that could be used to accelerate TCE dechlorination at OU5, and 4) detailed analysis of TCE interaction with Peerless ZVI and the effects of iron corrosion on resultant TCE degradation rates. The design of these studies is detailed below.

4.1 Culture Dilution Study

Two microbial cultures were included for evaluation in this study. One was a mixed, suspended growth culture enriched from a field site in Oscoda, Michigan, and the other was a granular mixed culture developed by MBI, Inc. Both cultures were provided to the UWRL by Retech in Lansing, Michigan, via a subcontract through MBI. The suspended growth culture, labeled the "Bachman Road" culture, was provided to the UWRL early in the study period, and was used to determine the maximum dilution that could be used in the microcosms to preserve rapid dechlorination rates with the minimum level of microbial innocula amendment.

Treatments for this study were constructed using OU5 site soil, nutrient solution, whey carbon donor, and TCE amended groundwater that included the following concentration of Bachman Road Culture: pure culture amendment (9 mL of pure Bachman Road Culture to 3 g soil in microcosm); 1:4 dilution of pure culture (dilution with TCE-amended OU5 groundwater); 1:10 dilution of pure culture; 1:100 dilution of pure culture; and 1:1000 dilution of pure culture. Each treatment consisted of triplicate reactors that were sampled at four to five sampling intervals over a 21-day incubation period. A summary of the treatment design for this experiment is provided in Table 4.1. Details of the construction, incubation, sampling and analysis of these reactors are provided in Section 5 – Materials and Methods, of this report, while results of this study are presented in Section 6 – Results and Discussion. The ultimate outcome of this study was the

Table 4.1. Design for the innocula dilution study using the Bachman Road Culture.

Treatment	Event 1 t = 0 days	Event 2 t = 3 days	Event 3 t = 7 days	Event 4 t = 14 days	Event 5 t = 21 days
Pure Culture	X	X	X	X	X
1:4 Dilution	X		X	X	X
1:10 Dilution	X		X	X	X
1:100 Dilution	X		X	X	X
1:1,000 Dilution	X		X	X	X

finding that a 1:10 dilution of microbial innocula was needed to ensure rapid TCE dechlorination in the OU5 microcosm systems.

4.2 Preliminary Range-Finding Study with MBI Granular Culture

The granular dechlorinating culture, labeled the "MBI Granular" culture, was used in this preliminary range-finding study to determine the relative TCE dechlorination rates provided by the culture when microcosms were augmented with a variety of carbon donors. Carbon donors used in this microbial amendment study were selected based on findings of the Hill AFB funded project evaluating carbon donor addition alone.

Treatments for this study were constructed using OU5 site soil, nutrient solution, whey and emulsified oil carbon donor, and TCE amended groundwater with a 1:10 dilution of the MBI Granular Culture. Both biotic and abiotic (autoclaved) microcosms were included in this study along with control reactors without carbon donor amendment. Each treatment consisted of triplicate reactors that were sampled at five sampling intervals over a 38-day incubation period. A summary of the treatment design for this experiment is provided in Table 4.2. Details of the construction, incubation, sampling and analysis of these reactors are provided in Section 5 – Materials and Methods, of this report, while results of this study are presented in Section 6 – Results and Discussion. The ultimate outcome of this study was the finding that a 1:10 dilution of the MBI Granular Culture was able to transform TCE completely to cis-DCE within a 2 week period, but that more than 38 days would be needed to ensure complete TCE dechlorination through VC and ethylene in the OU5 microcosm systems.

4.3 Complete Culture Augmentation Study

The full-scale Bachman Road and MBI granular culture microcosm augmentation study utilized the findings of the previous two experiments to establish reactor conditions that could be used to identify optimal microbial innocula and carbon donor combinations for the stimulation of TCE dechlorination in OU5 aquifer material. Once again, the carbon donors selected for use in this complete microbial amendment study were selected based on findings of the Hill AFB funded project evaluating carbon donor addition alone, and included a soluble donor, whey, an insoluble donor, coconut oil, and an emulsified

Table 4.2. Design for the range-finding carbon donor study using the MBI Granular Culture.

Treatment*	Event 1	Event 2	Event 3	Event 4	Event 5
Granule GW Control	3 days	7 days	13 days	26 days	38 days
Granule Soil Control	3 days	6 days	13 days	26 days	38 days
Granule + Whey	0 days	7 days	13 days	26 days	38 days
Granule + Emulsified Oil	0 days	6 days	13 days	26 days	38 days
ZVI	3 days	7 days	14 days	26 days	38 days

* GW Control = Granule + groundwater but no carbon donor,
Soil Control = Granule + OU5 soil + groundwater but no carbon donor.

donor, all which should the best performance of their respective donor classes in this Hill funded project.

Treatments for this study were constructed using OU5 site soil; nutrient solution; whey, coconut oil, and emulsified oil carbon donor; and TCE amended groundwater with a 1:10 dilution of the MBI Granular or Bachman Road cultures as their innocula amendment. Both biotic and abiotic (autoclaved) microcosms were included in this study, along with Zero Valent Iron treatment as a reactive, abiotic control; no carbon donor controls for each microbial innocula; and an aquifer control with neither carbon donor nor microbial innocula amendment. Each treatment consisted of triplicate reactors that were sampled at nine to 13 sampling intervals for organic analytes, and six sampling intervals for inorganic analytes over a 70 to 85-day incubation period. A summary of the treatment design for this experiment is provided in Tables 4.3 and 4.4. Details of the construction, incubation, sampling and analysis of these reactors are provided in Section 5 – Materials and Methods, of this report. The ultimate outcome of this study is the main emphasis of Section 6 – Results and Discussion of this report.

Table 4.3. Design of the complete culture augmentation study for inorganic analyte sample collection.

	Sampling Event (Time days)					
Treatment	**1**	**2**	**3**	**4**	**5**	**6**
Tmt A – Aquifer + GW Control	1	12	29	50	61	75
Tmt B – BR/No Donor	0	14	28	49	63	75
Tmt C – BR + Whey	0	7	14	22	48	79
Tmt D – MBI Granule/No Donor	0	14	28	49	63	84
Tmt E – MBI Granule + Whey	0	7	14	22	28	84
Tmt F – BR + Coconut Oil	0	7	14	21	31	72
Tmt G – MBI Granule + Coconut Oil	0	7	14	21	31	80
Tmt H – BR + Emulsified Oil	0	7	15	21	42	79
Tmt I – MBI Gran + Emulsified Oil	0	7	15	21	27	78
Tmt Z - ZVI	1	14	32	49	61	75

Table 4.4. Design of the complete culture augmentation study for organic analyte sample collection.

Treatment	Sampling Event (Time days, biotic/abiotic)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Tmt A – Aquifer + GW Control	0	7		15			25	28	37	42	48		75
Tmt B – BR/No Donor	0	7/ 8		15		21/ 22		28/ 29	36/ 37	41/ 42	53/ 54		75/ 76
Tmt C – BR + Whey	0	7/ 8	11	15	18/ 19	20/ 21	24/ 25	28/ 29	36/ 37	41/ 42	53/ 54	67/ 68	80/ 81
Tmt D – MBI Granule/No Donor	0	6/ 7		14/ 15			23/ 24	28/ 29	34/ 35	41/ 42	54/ 55		82/ 83
Tmt E – MBI Granule + Whey	0	6/ 7	14/ 15	17	19/ 20	23/ 24	25/ 26	28/ 29	34/ 35	41/ 42	54/ 55		82/ 83
Tmt F – BR + Coconut Oil	0	8/ 9	11	15/ 16	17/ 18	18	20/ 21	23/ 24	26/ 27	36/ 37	41/ 42	53/ 54	72/ 73
Tmt G – MBI Granule + Coconut Oil	0	7/ 6	11	14/ 15	18	19/ 21	23/ 24	28/ 29	36/ 35	41/ 42	53/ 55	67/ 68	80/ 81
Tmt H – BR + Emulsified Oil	0	7/ 8	11	14/ 16	18	21/ 22	23/ 24	28/ 29	36/ 37	41/ 42	53/ 54	67/ 68	76/ 77
Tmt I – MBI Granule + Emulsified Oil	0	8		16	22	26	29	34	38	41	54	69	79
Tmt Z - ZVI	0	7		15		25	28		37	42	48		75

4.4 Peerless ZVI-TCE Degradation Study

Iron metal (zero valent iron, Fe^{o}) has been shown to effectively remove chlorinated solvents, including TCE, from groundwater through oxidation-reduction reactions. ZVI has been used in subsurface permeable reactive barriers (PRB) and in aboveground treatment systems. The Fe^{o} is oxidized to Fe(II) or Fe(III) as TCE undergoes either sequential hydrogenolysis or β-elimination. With sequential hydrogenolysis, TCE forms DCE, vinyl chloride, ethene then ethane. With β-elimination, TCE is converted to chloroacetylene then to acetylene, ethene and ethane. The oxidation of iron leads to the formation of corrosion products of Fe(II) and Fe(III) precipitated with oxides, carbonates, etc. These corrosion products may influence the continued reactivity and/or permeability of the treatment system.

In this phase of the study the effect of corrosion of Peerless ZVI on the rate of TCE loss from groundwater collected from the northern plume of OU5 (since designated OU12) at Hill Air Force Base was evaluated. Corrosion product formation was evaluated using scanning electron microscopy. Preliminary studies were conducted to determine the relative reaction rates for the loss of TCE using two iron sources, stock iron fillings -8 to 50 mesh, Peerless Metal Powders and Abrasive, Inc. and iron fillings 40 mesh (surface area = 0.9 m^2/g) Fisher Chemical Co. The iron sources were pre-corroded under aerobic and anaerobic conditions to evaluate the effect of formation of iron corrosion products on TCE loss rate. Results for these preliminary studies are presented in the Appendix A. The final study design was developed from these results. Only Peerless ZVI was used in the present study, due to the high cost of using Fisher ZVI for any field application.

5. MATERIALS AND METHODS

5.1 Microcosm Construction and Incubation

5.1.1. Soil and groundwater sample collection. Soil and groundwater used in the construction of microcosms incubated in this study were provided through Hill AFB, EM, by their field sampling contractors, Montgomery-Watson. Soil samples were collected from an area approximately 1,500 ft downgradient of the aeration curtain in a location picked by Hill AFB, EM, where microbial innocula addition was thought to be feasible. Soil samples were collected from approximately 2 ft above groundwater to 5 ft below groundwater using a hallow stem auger, were place in plastic-lined 5-gal containers, were sealed, and were transported to the UWRL. Groundwater used in all experiments was collected from the Phase II groundwater extraction system (Figure 2.1) into 5-gal Neoprene laboratory containers. All soil and groundwater samples were stored at the UWRL at 4°C prior to use in the laboratory-scale microcosm systems.

5.1.2. Microcosm construction procedures. Details of microcosm construction are provided in Appendix B – SOP R-01 Standard Operating Procedures for Microcosm Construction, and are summarized here with emphasis on aspects of the procedure relevant to this study. Two types of microcosm systems were constructed during the course of this study to generate the data necessary to meet the study objectives. One reactor type was used for the analysis or specific organic constituents (TCE and its degradation products), and permanent gases, and consisted of 20-mL glass headspace vials with crimp-top seals and PTFE/Silicone septa. The other reactor type was used to provide samples for a variety of inorganic analyses and general dissolved organic carbon measurements, and were constructed from 125-mL glass bottle with PTFE/Silicone screw-top lids. Table 5.1 provides a summary of the general components of each reactor type based on the nature of carbon donor contained within a given reactor. Those reactors with microbial amendment addition were constructed in a manner identical to non amended reactors, with the exception that 10% of the reactor liquid volume in these microcosms was provided by the microbial innocula, 0.9 mL for the 20-mL reactors, and 7.5 mL for the 125-mL reactors. The Zero Valent Iron (ZVI) treatment was identical to

the unamended treatments except that 1 g/8.3 g of Peerless ZVI were added to the 20-mL and 125-mL reactors, respectively, prior to incubation.

Table 5.1. Summary of reactor components in microcosm experiments.

Reactors	Soil	Ground Water	Soluble Carbon	Yeast Solution	Insoluble Carbon
20 mL Carbon Donor Dechlorination Reactors (biotic or abiotic)	3.0 g Soil Dry Weight	8.8 mL TCE Amended (10 mg/L) Groundwater	200 µL	200 µL of 900 mg/L solution	0.6 g
20 mL Carbon Donor Dechlorination Reactors (biotic or abiotic) without Nutrient Admendment	3.0 g Soil Dry Weight	9.0 mL TCE Amended (10 mg/L) Groundwater	200 µL		0.6 g
125 mL Metals Release and Water Quality Indicator Reactors (biotic or abiotic)	25 g Soil Dry Weight	74.0 mL TCE Amended (10 mg/L) Groundwater	200 µL	1 mL of 1500 mg/L solution	5.0 g
125 mL Metals Release and Water Quality Indicator Reactors (biotic or abiotic) without Nutrient Admendment	25 g Soil Dry Weight	75.0 mL TCE Amended (10 mg/L) Groundwater	200 µL		5.0 g

To construct the microcosms, three sample of the site soil were obtained to determine a representative moisture content for the OU5 soil stored at the UWRL. A wet weight corresponding to the dry weights desired in the microcosms as indicated in Table 5.1 was then determined and used for reactor construction. This desired wet weight was placed into empty microcosms that had been washed and autoclaved prior to use in the experiments. Once the soil was placed in the reactors, the carbon donor was loaded into the microcosms by placing 0.2 mL of concentrated soluble donor solution, or 0.6/5.0 g of an inert carrier material (washed fine sand) loaded with 1 mg/g carrier of insoluble carbon donor (Coconut Oil) directly onto the soil in the microcosms. In the ZVI treatments 1.0 g/8.3 g of Peerless ZVI was added to the microcosms. Following donor or ZVI addition, the appropriate volume of nutrient solution (Table 5.1) was then added to the reactors using a repippette. If a reactor was to represent an abiotic system its top was then covered with aluminum foil and it was autoclaved for $\geq$ 15 minutes at 121°C, before being allowed to cool and receive further components of a complete treatment.

Microcosms with soil, carbon donor, and nutrient solution were then loaded with TCE amended groundwater from OU5. The groundwater placed within the reactors was first sparged with nitrogen for a minimum of 2 hours, was filter sterilized, and was then placed in 2-L glass bottles fitted with dispensing repipettes. To this sparged and filter sterilized groundwater was added an aliquot of a concentrated TCE solution prepared by equilibrating 30 mL of OU5 groundwater with 10 mL of neat TCE in a sealed 40-mL

VOA vial to yield an initial TCE concentration in the microcosms of approximately 10 mg/L. As indicated above, for those microcosms receiving microbial innocula, specified fractions of the reactor liquid volume were replaced by the microbial culture. Abiotic treatments were amended with aliquots of filter sterilized innocula medium. Following addition of the requisite volume of groundwater/innocula/filter sterilized innocula, the reactors were sealed and stored for the appropriate time period at 15°C before being sacrificed for sample collection and analysis.

To ensure strict anaerobic conditions within the microcosms during incubation, once the soil, carbon donor, and nutrient solutions were added to the reactors they were placed within an anaerobic chamber with their lids lightly sealed for at least 4 hours before groundwater and innocula were added to them. In addition, the TCE/groundwater solution was prepared, the TCE amended groundwater and microbial innocula were added, and the microcosms were sealed in the anaerobic glove box. To further minimize leakage of TCE and degradation products from the reactors, they were inverted during their incubation time in the anaerobic chamber.

5.2 Microcosm Sampling and Analytical Procedures

At each of the sampling intervals indicated in Tables 4.3 and 4.4, triplicate reactors for a given treatment were sacrificed for sampling and analysis. Specific sampling and analysis procedures used were dependent upon the type of reactor being sampled, and are described separately below.

5.2.1. Headspace organic analysis reactors. The 20-mL microcosm reactors were removed from the anaerobic glove box, were immediately transferred to the analytical laboratory, and were placed in a sample carrousel in preparation for headspace analysis of VOCs and permanent gases using the methods outlined in Table 5.2. Detailed SOPs for these analytes are found in Appendixes C and D of this report.

Table 5.2 Specific organic analytes, method detection limits (MDLs), and analytical methods used for their quantification during the laboratory microcosm studies.

Analyte Class	Specific Analyte	Sample Injection	MDL	Analysis Method
Halogenated Organics	TCE, cis-/trans-DCE, VC	Headspace, SW846 5021	0.65, 0.61/0.91, 1.1 µg/L	GC/MS, U.S. EPA, SW846 8260B
Permanent Gases	Ethylene, Methane, Ethane	Headspace, SW846 5021	5.1, 9, 4.8 ppm_v	GC/FID, Supelco (1996)
Permanent Gases	CO_2, H_2S	Headspace, SW846 5021	0.225, 0.05, vol%	GC/TCD, Supelco (1996)

5.2.2. Water quality analysis reactors. The 125-mL microcosm reactors were sampled and analyzed for arsenic, terminal electron acceptors, pH, EC, DOC and ORP over the course of the study. At the appropriate sampling time, these reactors were opened within an anaerobic glove box under a pure nitrogen environment and a 5 mL sample was immediately analyzed for Fe(II) using a ferrozine method developed by Lovely and Phillips (1986). In addition, a 5 mL sample was passed through a resin column and into a 10-mL polyethylene container acidified with nitric acid to pH $\leq$ 2 for As (III) analysis according to the method of Wilkie and Hering (1998). The remaining sample was removed from the glove box for filtering and non-redox sensitive analyses.

Outside of the glove box, the samples were directly analyzed for DO and ORP using electrometric methods by placing these analyte probes directly into the microcosms with the probe tips positioned immediately above the top of the sediment layer in each reactor. Once these analyses were completed 10 mL of the sample were transferred into a 100 mL glass test tube in which pH and EC were immediately measured. Finally, the remaining sample was filtered through a 0.45 µ filter, and the filtrate was immediately transferred to sample containers as follows: 1) 5 mL into polyethylene sample containers for sulfate and nitrate/nitrite analyses; 2) 5 mL into 40-mL amber glass VOA containers acidified with phosphoric acid to pH $\leq$ 2 and then sparged for DOC analyses; and 3) 30 mL into a 30-mL polyethylene snap-cap container acidified with nitric acid to pH $\leq$ 2 for total dissolved arsenic, iron, and manganese analyses. All analyses were carried out using the standard methods identified in Table 5.3 and SOPs outlined in the Appendixes E to K.

5.3 ZVI-TCE Degradation Study Methods

Three types of ZVI were utilized in the ZVI-TCE degradation study: 1) untreated Peerless ZVI, 2) anaerobically pre-corroded Peerless ZVI, and 3) aerobically pre-corroded ZVI. For the pre-corroded studies, 8 g of Peerless ZVI were added to 22-mL glass vials and 8-mL of filtered-sterilized uncontaminated OU5 (OU12) groundwater were added. The anaerobic corroded samples were processed and shaken in the anaerobic glovebox. Aerobic samples were filled with aerated groundwater and shaken (open to the atmosphere) in the lab. Samples were shaken for 6 weeks to promote corrosion. After this time period, corrosion products were visible under both corrosion regimes.

Kinetic studies were then conducted by adding 8 g of untreated Peerless ZVI to 22-mL glass vials. For the ZVI samples that had been pre-corroded, the water was siphoned-off leaving the corroded iron in the original vials. Uncontaminated groundwater collected from OU5 (OU12) was filtered-sterilized and augmented with TCE. Targeted concentration for the spikes was 300 to 500 µg/L for TCE. The initial concentrations of TCE for each study are listed in Table 5.3. Eight mL of the augmented water were added to each reaction vial, the vials were sealed with Teflon septa, and were then shaken on a reciprocal shaker at 100 rpm. Control samples were set-up without ZVI. All sample processing and storage of the anaerobically pre-corroded and untreated ZVI was

conducted under anaerobic conditions using a N_2 glovebox. Aerobically corroded samples were processed and stored in the lab. Samples were analyzed in triplicate at time

Table 5.3 Specific inorganic analytes, method detection limits (MDLs), and analytical methods used for their quantification during the laboratory microcosm studies.

Analyte Class	**Specific Analyte**	**Sample Injection**	**MDL**	**Analysis Method**
Water Quality Parameters	Sulfate, Nitrate/Nitrite	Direct Injection	0.1, 0.02, 0.02 mg/L	IC, U.S. EPA300.0
	Organic Carbon	Syringe Injection	0.12 mg/L	Persulfate/UV, U.S. EPA415.1
	pH	N/A	N/A	Electrometric, U.S. EPA150.1
	EC	N/A	N/A	Electrometric, U.S. EPA120.1
	ORP	N/A	N/A	Electrometric, APHA2580
Metals	Fe(II)	N/A	12 µg/L	Colorometric w/Ferrozine, Lovely & Phillips (1986)
	As(III)	Direct Injection following Resin Separation	0.8 µg/L	GFAA, 7060A U.S. EPA SW846
	Total As, Fe, Mn	Direct Injection	0.8, 60, 30 µg/L	GFAA, 7060A, FAA 7380, and 7460, respectively, U.S. EPA SW846

Table 5.4. Initial concentration of TCE utilized in the ZVI-TCE degradation experiments.

Treatment	Initial TCE Concentration (µg/L)
Groundwater + untreated ZVI	440
Groundwater + anaerobic corroded ZVI	120
Groundwater + aerobic corroded ZVI	440

0, 4, 8, 12, 24, 48, 72, 96, 180, 330 hours. TCE and chlorinated breakdown products were analyzed within the reaction vials using a headspace analyzer and GC-MS as described above.

After the analysis for TCE, sample vials from the initial and final time intervals were put in the glove box. Water was poured out and the ZVI with associated corrosion products was dried in the glove box (nitrogen atmosphere) for 2 days. The dried iron samples were then transferred to a VOA vial and were held at room temperature awaiting scanning electron microscope (SEM) analysis. This SEM technique is widely applied in microbiology and mineralogy to produce magnified three-dimensional images of a sample matrix. Secondary and backscattered electron images can be generated by different electrons in sample atoms that react with scanning electron beams. Secondary electrons are used to generate an image based on topographic contrast, i.e., three-dimensional features of a sample, whereas backscattered electrons are generated based on different atomic numbered elements (Z contrast) within a sample. Higher atomic numbered elements appear slightly brighter than lower atomic numbered elements in a secondary electron detector and significantly brighter in a backscattered electron detector (Bozzola and Russell, 1991). Energy dispersive X-ray (EDX) analysis is also routinely used in conjunction with SEM analysis for the acquisition, sorting, and display of data as a spectrum of energies. A peak on the spectrum corresponds exclusively to a different atom shell of a specific atom, which can be used to identify elements contained in the portion of sample that is scanned by the beam.

Prior to SEM analysis of the samples generated in this study, vials were shaken to ensure complete mixing, and one randomly chosen aliquot of each sample was taken out of the VOA vial for analysis. Three to six different spots on the chosen portion of iron sample were analyzed by SEM with secondary and backscatter detectors and EDX. A Hitachi S-4000 Scanning Electron Microscope was used to accomplish these analyses. Samples were analyzed in the SEM lab within 7 days to minimize the opportunity for oxygen diffusion into the VOA vials and impacts on corrosion products in the iron samples.

5.4 Data Reduction Procedures

Raw analytical data were converted to appropriate concentration or unit terms using data reduction methods specified in the SOPs contained in Appendixes A to J. These analytical procedures generally use calibration curves or internal instrument calibration to provide a quantitative measure of analyte concentration in a sample. These data were then entered and manipulated in Excel spreadsheets to provide plots of parameter changes over time within and between reactor treatments.

The concentrations of TCE and its daughter products were converted to molar concentration values by dividing mass concentration data generated from analytical results by the molecular weight of each compound. These molar concentration data were then normalized to the initial concentration of each treatment by dividing the

concentrations measured at each time interval by the mean of the initial concentration for each compound. The natural log transformation of these normalized molar concentrations were then plotted versus time to generate an estimated pseudo-first order rate constant observed for each treatment during the course of the study. The general equation for a first order degradation rate expression is shown in Equation 1,

$$dC/dt = -k_1 C \quad (1)$$

which is integrated to yield the final form of:

$$\ln(C/Co) = -k_1 t \quad (2)$$

With TCE degradation being a sequential reduction reaction to cis-DCE, VC and ethylene, a series of pseudo first-order reactions can be described, yielding a series of first order reaction rates that can be estimated from TCE and intermediate compound data measured over time from the reactors. This series of reactions can be approximated as follows:

$$\text{TCE} \xrightarrow{k_1} \text{cis-DCE} \xrightarrow{k_2} \text{VC} \xrightarrow{k_3} \text{ethylene} \quad (3)$$

and individual contaminant concentrations can be predicted over time using the following finite-difference expressions:

TCE	$C_t = C_{t-1} (1 - k_1 \Delta t)$	(4)
cis-DCE	$C_t = C_{t-1} (1 - k_2 \Delta t) + TCE_{t-1} (k_1 \Delta t)$	(5)
VC	$C_t = C_{t-1} (1 - k_3 \Delta t) + \text{cis-DCE}_{t-1} (k_2 \Delta t)$	(6)
ethylene	$C_t = C_{t-1} + VC_{t-1} (k_3 \Delta t)$	(7)

The total number of moles of TCE and its degradation products should be conserved in the microcosms, leading to a final relationship used in the analysis of the study data as shown in Equation 8:

$$\sum (\text{TCE} + \text{cis-DCE} + \text{VC} + \text{ethylene}) = \text{constant in molar terms} \quad (8)$$

6. RESULTS AND DISCUSSION

6.1 Culture Dilution Study

The purpose of this phase of the study was to evaluate the effect of microbial culture dosing concentration on the measured TCE dechlorination activity in the microcosm systems. The Bachman Road Culture was used undiluted (9 mL of Bachman Road Culture to 3 g soil in microcosm), as a 1:4 dilution (dilution with TCE-amended OU5 groundwater), as a 1:10 dilution, as a 1:100 dilution, and as a 1:1,000 dilution in the microcosm treatments. TCE and its degradation products were monitored periodically over a 21-day incubation period, and the mass and molar concentration of these

chlorinated hydrocarbons were evaluated to select the optimal dilution to use in subsequent microcosm studies.

Figure 6.1 shows the final results of TCE mass concentrations measured in the reactors of this culture dilution study. All raw data for this experiment are included in Appendix K. Initial TCE concentrations used here were slightly higher than desired due to the high TCE feed concentration (25 mg/L) used in growing the culture. In addition, measurable concentrations of TCE daughter products were observed in the t=0 samples due to the level of these breakdown product in the pure culture growth medium when it was added to these microcosms. The concentration of these daughter products, cis-DCE and VC, for all culture dilutions, and for all sampling events are summarized in Figures 6.2 and 6.3, respectively.

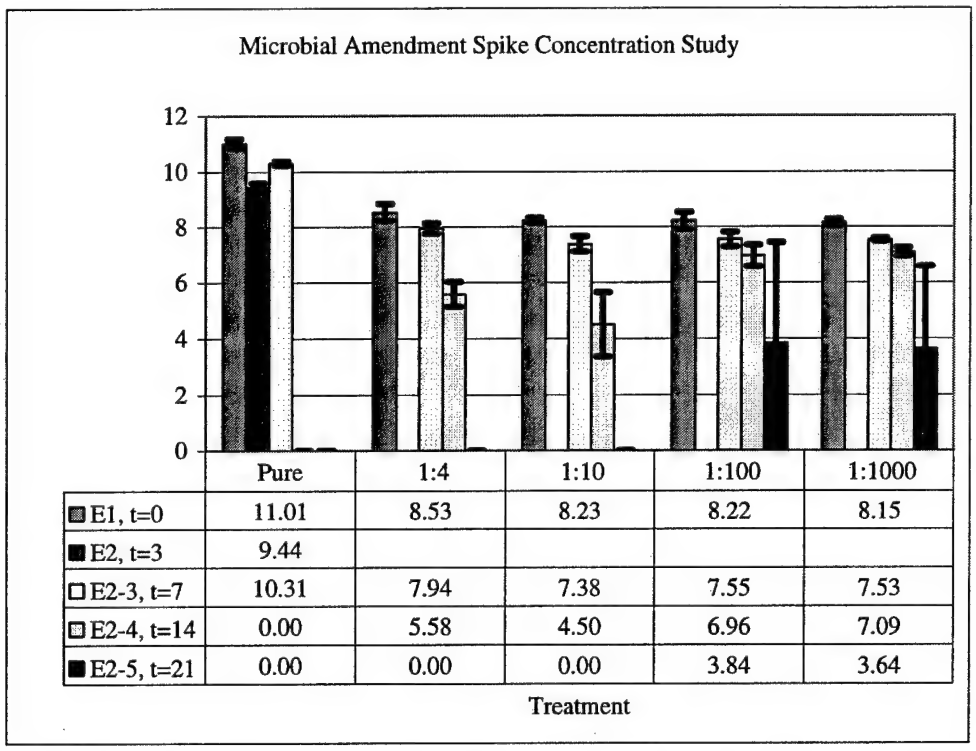


Figure 6.1. Variation of TCE mass concentrations over time in the Bachman Road Culture-amended microcosms of various culture dilutions. Note bars represent 95% Confidence Intervals of the measurement.

Significant transformation of TCE was observed in all treatment dilutions, with TCE transformation rates decreasing with increasing culture dilution. TCE removal was complete in the pure culture system by Day 14, while it was complete by Day 21 in the 1:4 and 1:10 dilutions. Approximately 50% TCE degradation was observed in the 1:100 and 1:1,000 dilutions by the end of the study at Day 21. Degradation products of TCE were observed in all treatments as indicated in Figures 6.2 and 6.3, with DCE peaking and significant VC production being observed in the pure culture dilution by Day 14 of the study. All culture dilutions produced significant (mg/L) levels of cis-DCE by the end of the study period, although VC was only observed in the pure dilution systems.

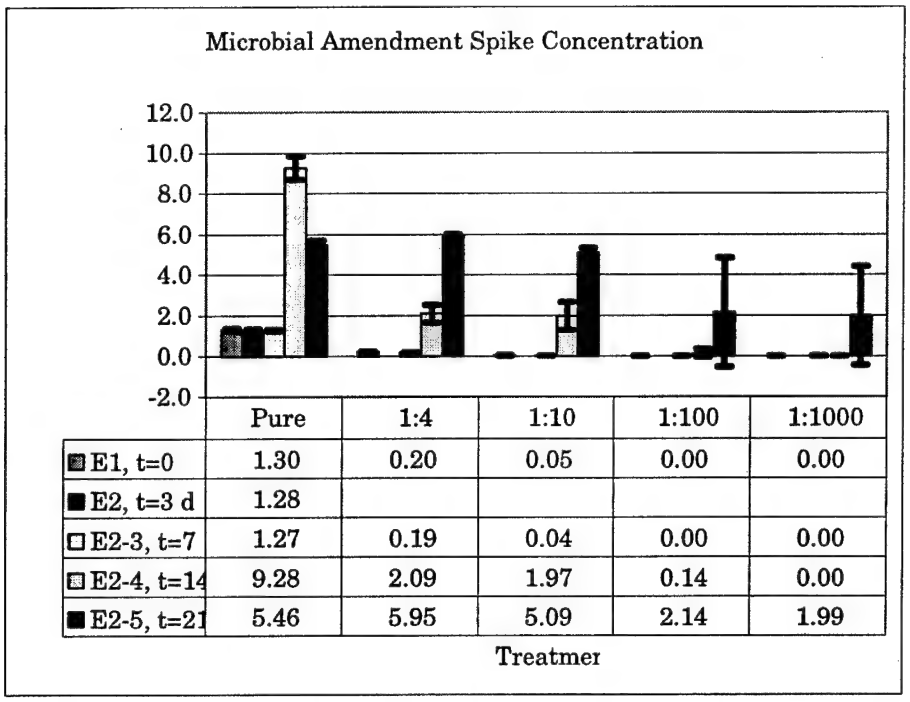


Figure 6.2. Variation of cis-DCE mass concentrations over time in the Bachman Road Culture-amended microcosms of various culture dilutions. Note bars represent 95% Confidence Intervals of the measurement.

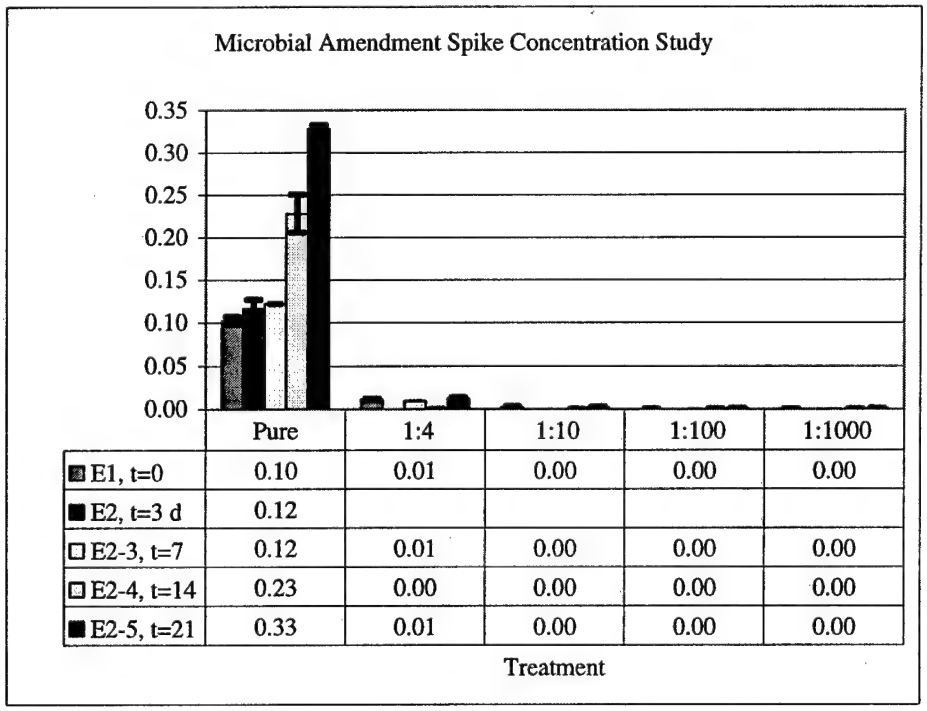


Figure 6.3. Variation of VC mass concentrations over time in the Bachman Road Culture-amended microcosms of various culture dilutions. Note bars represent 95% Confidence Intervals of the measurement.

Figure 6.4 shows the molar balance for chlorinated alkene species within the Bachman Road dilution study reactors, and Table 6.1 summarizes the results of this study in terms of degradation product relationships over time. Significant conversion of all chlorinated alkenes to ethylene and carbon dioxide was only found to occur in the undiluted treatment within the 21-day incubation period. Due to economic considerations related to future scale-up, a 1:10 dilution was selected for use in further microcosm studies as it was the highest dilution that yielded the desired degradation relationship (Table 6.1) producing an acceptable chlorinated hydrocarbon degradation rate.

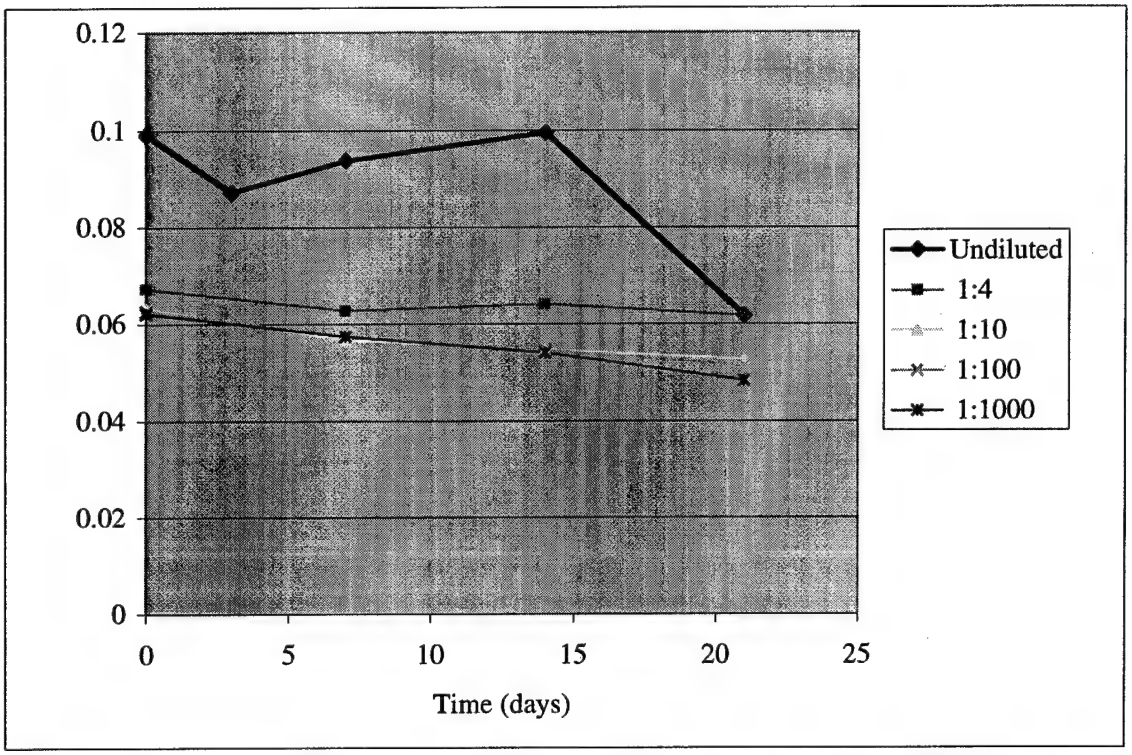


Figure 6.4. TCE, cis-DCE, and VC milimolar balance over time in the Bachman Road dilution study.

Table 6.1 Summary of results from Bachman Road dilution study.

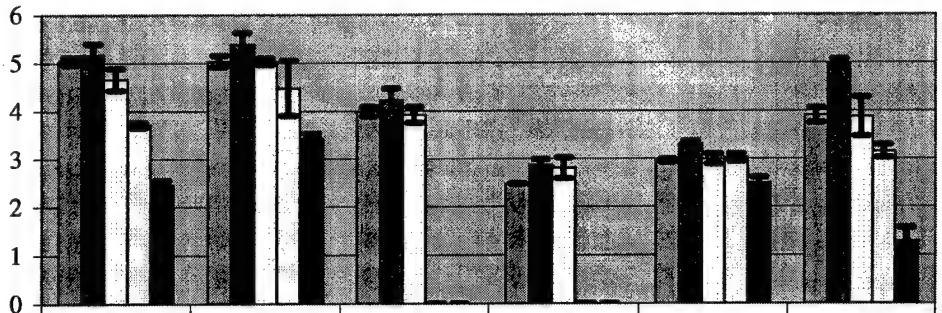
Dilution	Time to 0 TCE	Time to Produce cis-DCE	Time to Peak cis-DCE	Peak cis-DCE (mg/L)	Time to Produce VC	Time to Peak VC
Pure	7-14	7-14	14	9.3	7-14	> 21
1:4	14-21	7-14	≥ 21	5.95	> 21	>> 21
1:10	14-21	7-14	≥ 21	5.09	> 21	>> 21
1:100	> 21	14-21	≥ 21	2.14	> 21	>> 21
1:1000	> 21	14-21	≥ 21	1.99	> 21	>> 21

6.2 Preliminary Range-Finding Study with MBI Granular Culture

The purpose of this phase of the study was to determine the relative TCE dechlorination rates provided by the MBI Granular Culture when microcosms were augmented with a variety of carbon donors, and to assess the rate of ZVI abiotic transformation of TCE in laboratory reactors. Whey and an emulsified oil were used as carbon donors and were dosed at approximately 1,000 mg/L carbon at the beginning of study. Each reactor was inoculated with the a 1:10 dilution of the MBI Granular Culture (0.9 mL MBI Granular Culture to 8.1 mL TCE amended groundwater) based on the Bachman Road culture dilution experiment results presented above. No water quality parameter reactors were constructed for this preliminary run. TCE and its degradation products were monitored periodically over a 38-day incubation period in both biotic and abiotic reactors, and the mass concentration of these chlorinated hydrocarbons were evaluated to verify transformation of TCE during incubation.

Final TCE and cis-DCE concentration data from these systems in the biotic reactors are summarized graphically in Figures 6.5 and 6.6, respectively. All raw data for this experiment are included in Appendix L. TCE removal was complete in the Granule plus whey and emulsified oil carbon donor systems by Day 26, while no TCE transformation was observed without donor addition (Treatments Gran A or Gran B), or without microbial amendment (Treatment X). Also, no TCE transformation was observed in any of the abiotic treatments except for the ZVI amended reactors (Treatment Z). Transformation in the ZVI treatment microcosms, regardless of their biological status, is expected based on the abiotic nature of ZVI reaction with TCE. Some TCE transformation in the ZVI amended microcosms was apparent by Day 38 based on a significant reduction in TCE concentration and small levels of cis-DCE as a degradation product (Figure 6). In the two Granule + Donor treatments, DCE was shown to peak by Day 26, but was not completely metabolized by the end of the study. Small concentrations of VC (42 µg/L) were evident in the Gran X treatment, but no VC was detected over time in the Granule plus whey reactors. A summary of TCE and intermediate product formation results for the MBI Granular amendment study is provided in Table 6.2. These results are consistent with the previous findings of the Bachman Road Culture dilution study, and indicated that a 1:10 dilution of the MBI Granular Culture was sufficient to ensure rapid TCE degradation rates in the laboratory microcosm reactors.

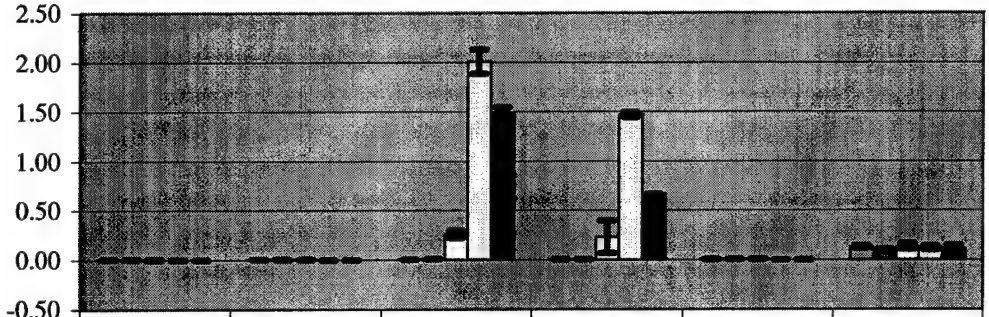
TCE transformation rates were estimated for the Soil Control (Treatment A), both biotic and abiotic treatments, and ZVI amended (Treatment Z) microcosms to generate an estimate of the magnitude of the abiotic reaction rate of TCE with ZVI in these laboratory systems, corrected for background losses during incubation. Figure 6.7 shows the normalized TCE concentration data along with the results of the linear regression of each data set. As can be seen from this figure, biotic and abiotic losses in the Soil Control reactors were identical, suggesting that over the course of this 38-day study, only abiotic losses due to time variant sorption, leakage, etc., were evident from the incubating microcosms. The ZVI treatment showed significantly greater TCE removal over time, with an apparent TCE reaction rate of –0.0016/hr, and a control-corrected rate of



	Gran A - GW Control	Gran B - Soil Control	Gran C - Whey + Bug	Gran X - Emul Oil + Bug	X - Emulsified Oil	Z - ZVI
E1, t=0	5.0	5.03	4.0	2.47	2.95	3.90
E2, t=6d	5.2	5.39	4.2	2.86	3.30	4.96
E3, t=13d	4.7	5.02	3.9	2.80	2.98	3.87
E4, t=26d	3.7	4.47	0.0	0.00	3.02	3.15
E5, t=38d	2.5	3.42	0.0	0.00	2.49	1.28

Treatment

Figure 6.5. Variation of TCE concentrations over time in MBI Granular Culture study biotic microcosms. Note bars represent 95% Confidence Intervals of the measurement.



	Gran A - GW Control	Gran B - Soil Control	Gran C - Whey + Bug	Gran X - Emul Oil + Bug	X - Emulsified Oil	Z - ZVI
E1, t=0	0.00	0.00	0.00	0.00	0.00	0.13
E2, t=6d	0.00	0.00	0.01	0.00	0.00	0.09
E3, t=13d	0.00	0.00	0.26	0.23	0.00	0.13
E4, t=26d	0.0	0.00	2.0	1.47	0.00	0.12
E5, t=38d	0.0	0.00	1.5	0.66	0.00	0.11

Treatment

Figure 6.6. Variation of cis-DCE concentrations over time in MBI Granular Culture study biotic microcosms. Note bars represent 95% Confidence Intervals of the measurement.

Table 6.2 Summary of results from MBI Granular Culture range-finding study.

Treatment	Time to 0 TCE	Time to Produce cis-DCE	Time to Peak cis-DCE	Peak cis-DCE (mg/L)	Time to Produce VC	Time to Peak VC
Gran A – GW Control	>> 38 days	>> 38 days	>> 38 days	N/A	>> 38 days	>> 38 days
Gran B – Soil Control	>> 38 days	>> 38 days	>> 38 days	N/A	>> 38 days	>> 38 days
Gran C – Whey + Bug	13-26 days	0-6 days	13-26 days	≥ 2.0	N/A	N/A
Gran X – Emulsified Oil + Bug	13-26 days	6-13 days	13-26 days	≥ 1.47	> 21 days	≥38 days
X – Emulsified Oil	>> 38 days	>> 38 days	>> 38 days	N/A	N/A	N/A
Z - ZVI	>38 days	6-13 days	6-13 days	≥ 0.13	N/A	N/A

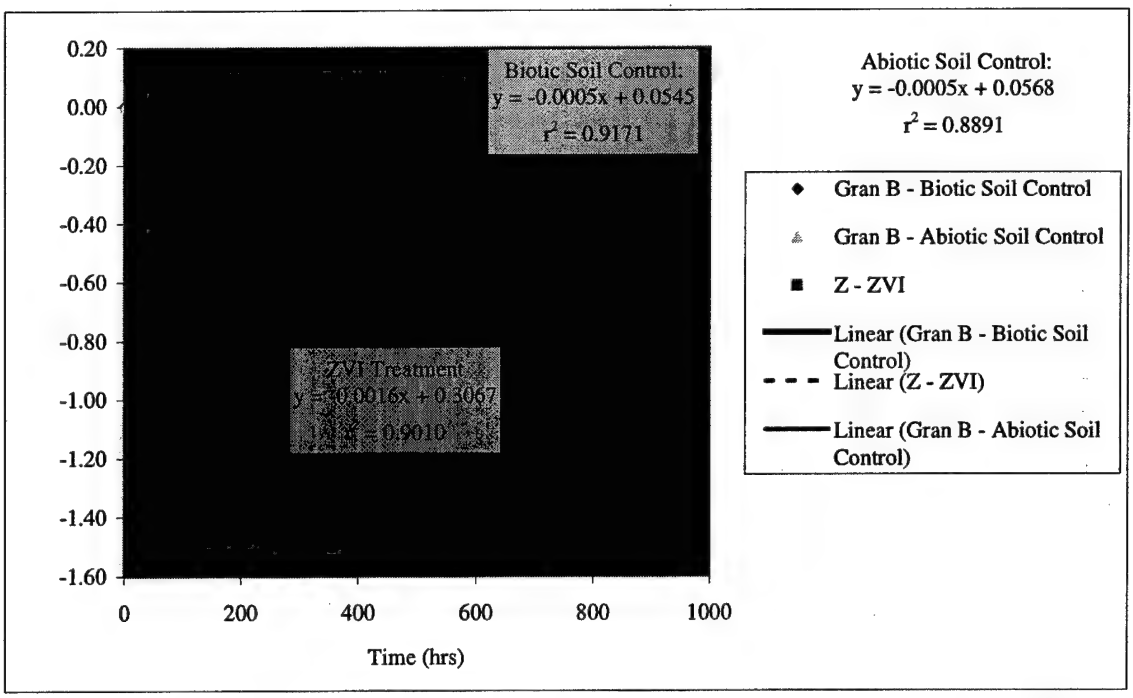


Figure 6.7. Linear regression results for normalized TCE concentrations from MBI Granular Culture range-finding study, Treatment B – Soil Control, and Treatment Z – ZVI amended reactors.

–0.0011/hr. This control-corrected rate corresponds to a TCE half-life of 642 hr or 26.7 days, and is consistent with other reported TCE-ZVI reaction rates in batch reactor systems.

6.3 Complete Culture Augmentation Study

The purpose of this final phase of the study was to quantitatively determine the relative TCE and daughter product dechlorination rates provided by the two microbial cultures under investigation in this study when microcosms were augmented with a variety of carbon donors, using ZVI abiotic transformation of TCE as a positive, abiotic control in the microcosm systems. Whey, coconut oil, and an emulsified oil were used as carbon donors in the study. The soluble and emulsified oil donor were dosed at approximately 1,000 mg/L carbon, while the coconut oil was dosed at approximately 150 mg/L carbon at the beginning of study. Each bioaugmented reactor was inoculated with the a 1:10 dilution of a particular culture (0.9 mL culture to 8.1 mL TCE amended groundwater), and TCE and its degradation products were monitored periodically over a maximum 85-day incubation period in both biotic and abiotic reactors. Water quality parameter reactors were constructed for this complete run., and the mass and molar concentration of these chlorinated hydrocarbons were evaluated to verify transformation of TCE, evaluate molar balances, and to generate estimated degradation rates for data collected during reactor incubation. Data were generated for reactors without bioaugmentation in addition to those that were bioaugmented, and the results of these independent reactor treatments are presented separately below before being compared at the end of this section.

6.3.1. Degradation results from non-bioaugmented reactors. Final TCE molar concentration data from these systems in the biotic reactors are summarized graphically in Figure 6.8. All raw chlorinated hydrocarbon data for this experiment are included in Appendix M. Only sporadic and very low levels of TCE transformation products were observed in any of these carbon donor treatments over the course of the incubation period. Pseudo first-order TCE loss rates were estimated based on the normalized TCE concentration data presented in Figure 6.8, and Figures 6.9 and 6.10 present examples of these regression results. Table 6.3 summarizes the estimated pseudo first-order loss rates and the control-corrected TCE degradation rates observed in these carbon-only amended reactors. As indicated in Table 6.3, once TCE loss rates were corrected for TCE concentration reductions observed in the Soil Control reactors, only the Whey amended reactors showed TCE loss significantly greater than the control treatments. Without bioaugmentation, the TCE degradation rate in the Whey amended reactors was only –0.0025/d, corresponding to a TCE half-life in these systems of approximately 40 weeks, an unacceptably low degradation rate for application in a site remediation system.

Water quality data were also collected from these non-bioaugmented reactors over time to assess the impact of carbon donor addition on water quality in the microcosms. Raw data for these general water quality parameters are located in Appendix N. Microcosm pH and electrical conductivity showed little variation over time, or as a function of biological status within a given reactor treatment during these microcosm studies. Microcosm pH averaged over all treatments, all time intervals and for biotic and abiotic reactors, averaged 7.8, with a range of observed values from pH 5.6 to 8.9. Microcosms amended with high carbon dose levels tended to have the lowest pH values, but pH even in these reactors reached neutral to slightly alkaline pH levels by the end of

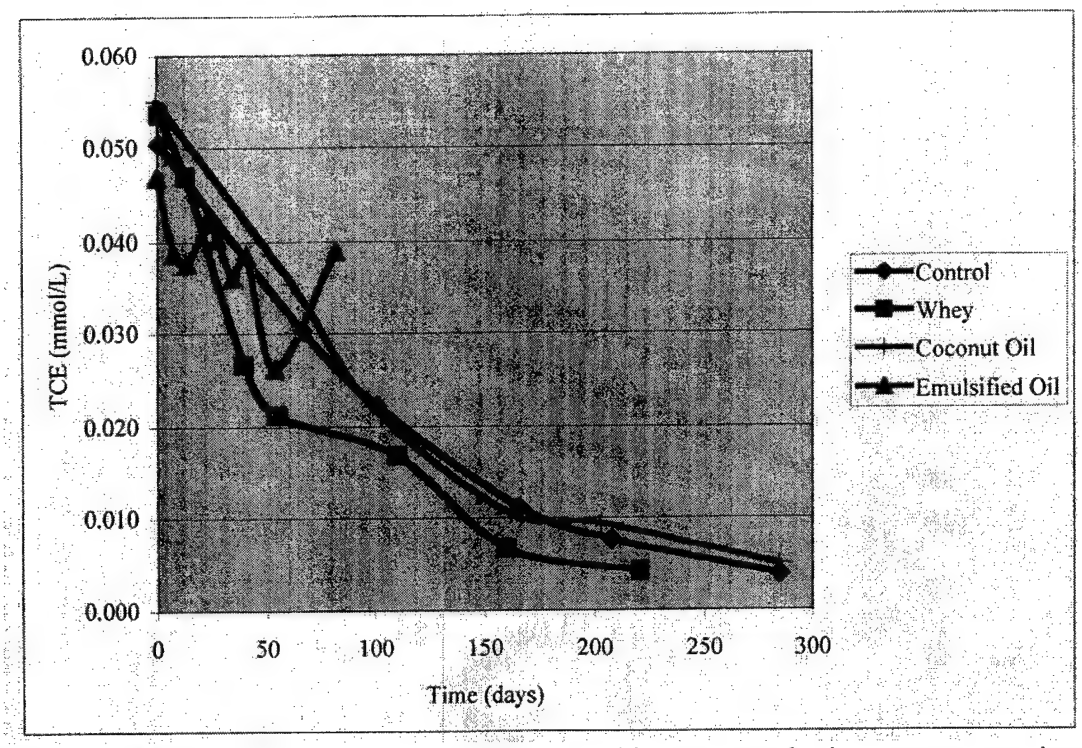


Figure 6.8. Molar TCE concentrations in the non-bioaugmented microcosms over time as a function of carbon donor amendment.

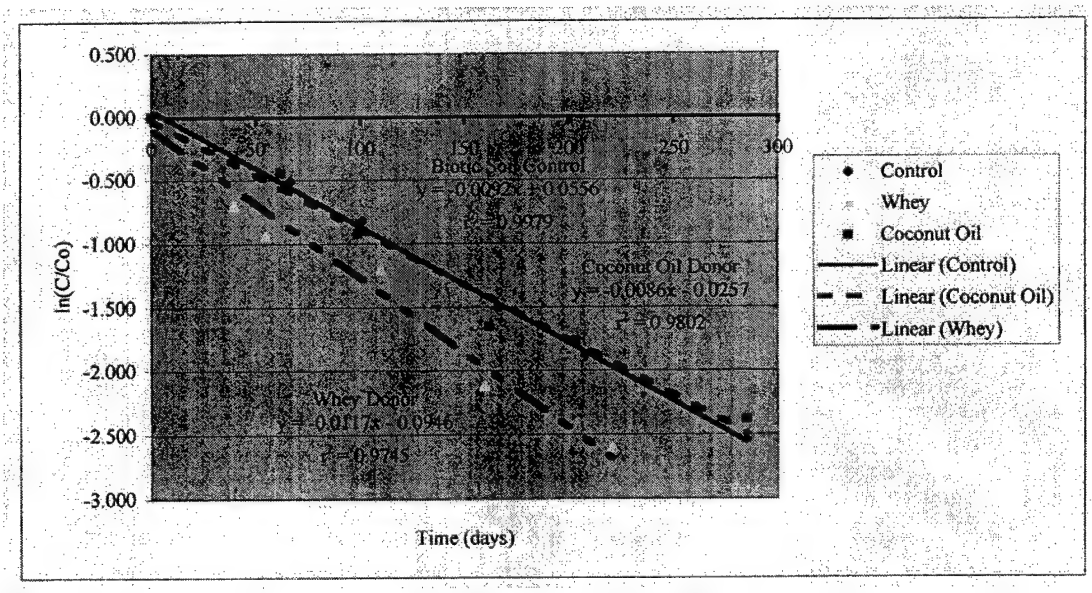


Figure 6.9. Linear regression results for normalized TCE concentrations for select non-bioaugmented microcosms.

Table 6.3 Summary of estimated pseudo fist-order TCE loss, and control corrected first-order TCE degradation results from the non-bioaugmented, carbon donor-amended reactors.

Treatment	TCE Loss Rate, 1/day	Control Corrected TCE Degradation Rate, 1/d
Control	-0.0092	
Whey	-0.0117	-0.0025
Coconut Oil	-0.0086	No Degradation
Emulsified Oil	-0.0030	No Degradation

the study as indicated by a mean pH across all reactors of 8.1 and a pH range of 6.95 to 8.92 for individual reactors measured during the last sampling interval. Electrical conductivity values varied significantly as a function of carbon donor type, with the Whey amended reactor reaching EC levels of over 3 mS/cm as this substrate was converted to volatile fatty acids during incubation. The Control and Coconut Oil amended reactors produced an EC level of approximately 1 mS/cm that slowly decreased to approximately 0.5 mS/cm by the end of the study. The Emulsified Oil treatment produced an intermediate EC level, averaging 1.4 mS/cm and only ranging from 1.1 to 1.8 mS/cm during the study.

Reactor oxidation reduction potential (ORP) was much more variable both within and among treatments over time than pH or EC as indicated in Figure 6.10. Some depression of ORP was evident in the Whey and Emulsified Oil treatments as substrate metabolism took place in these reactors, but reducing conditions were only predominant with the Emulsified Oil carbon donor.

Electron acceptor composition changes should also reflect microbial metabolism taking place during incubation, and dissolved iron and sulfate concentrations in the non-bioaugmented reactors over time are shown in Figures 6.11 and 6.12, respectively. Nitrate concentrations were reduced to non-detectable levels after the initial sampling event, and measurable nitrate concentrations were not measured again during the study. The concentration profile for dissolved iron and sulfate indicate significant levels of iron reduction in the Whey and Emulsified Oil amended reactors, and complete sulfate reduction in all of the carbon donor treatments within 30 to 50 days of the start of incubation. These microcosm systems were clearly reduced by the addition of carbon donor despite the ORP data indicating relatively oxidized conditions even in the donor amended treatments.

This reducing capacity was provided through the addition of carbon, and the change in carbon concentration in these reactors over time is indicated in Figure 6.13. The truly soluble donor, Whey, was amended at an approximate concentration of 1,000 mg/L carbon, and this carbon level persisted through the fourth sampling event at 168 days of incubation before falling to 25 mg/L carbon through the balance of the 280 day incubation period. The low solubility donor, Coconut Oil showed some increase in

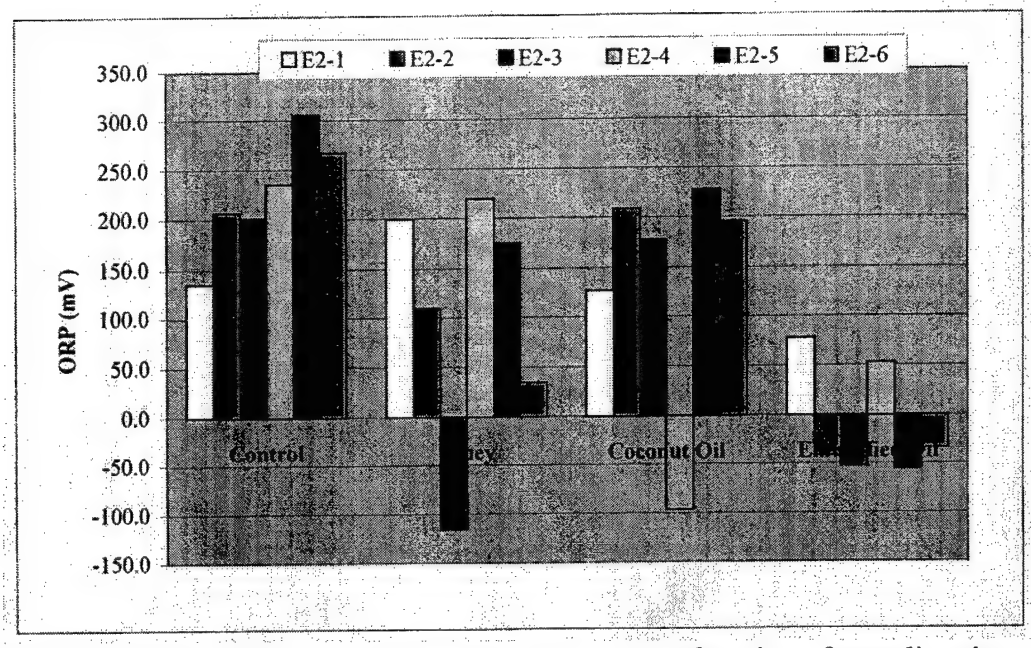


Figure 6.10. Mean ORP measured in biotic reactors as a function of sampling time and carbon donor for non-bioaugmented microcosms.

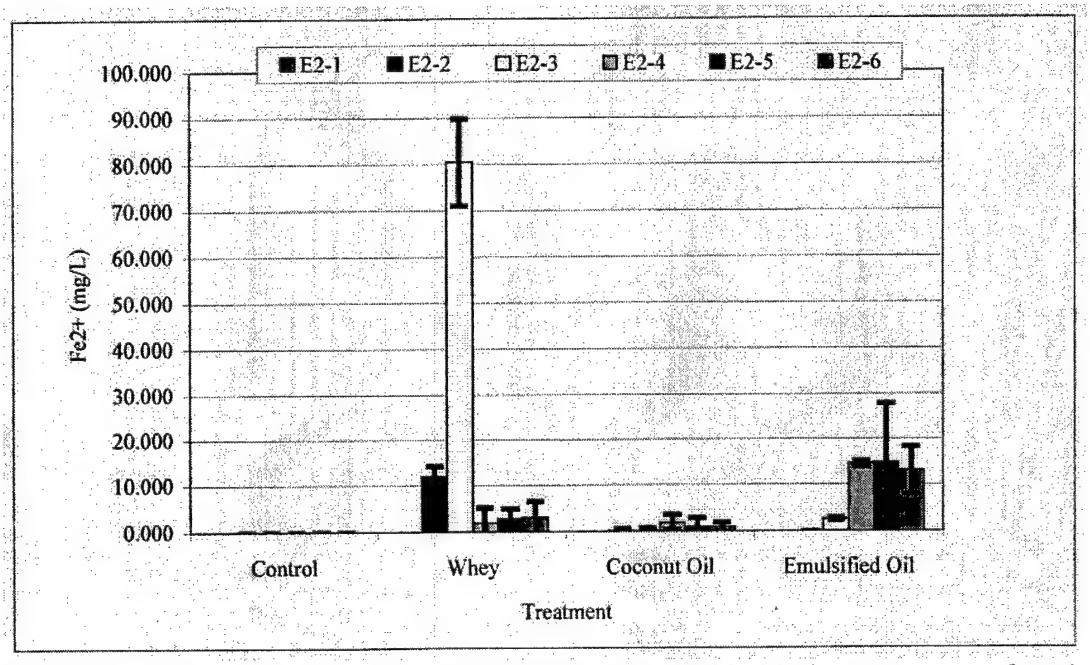


Figure 6.11. Mean dissolved iron concentrations measured in biotic reactors as a function of sampling time and carbon donor for non-bioaugmented microcosms.

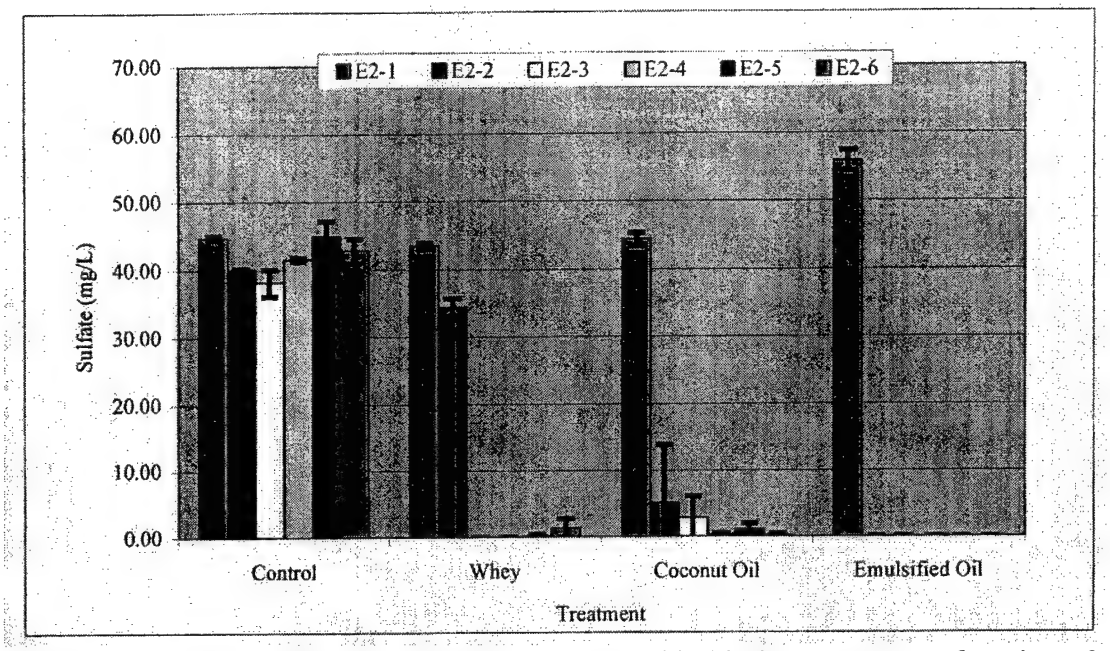


Figure 6.12. Mean sulfate concentrations measured in biotic reactors as a function of sampling time and carbon donor for non-bioaugmented microcosms.

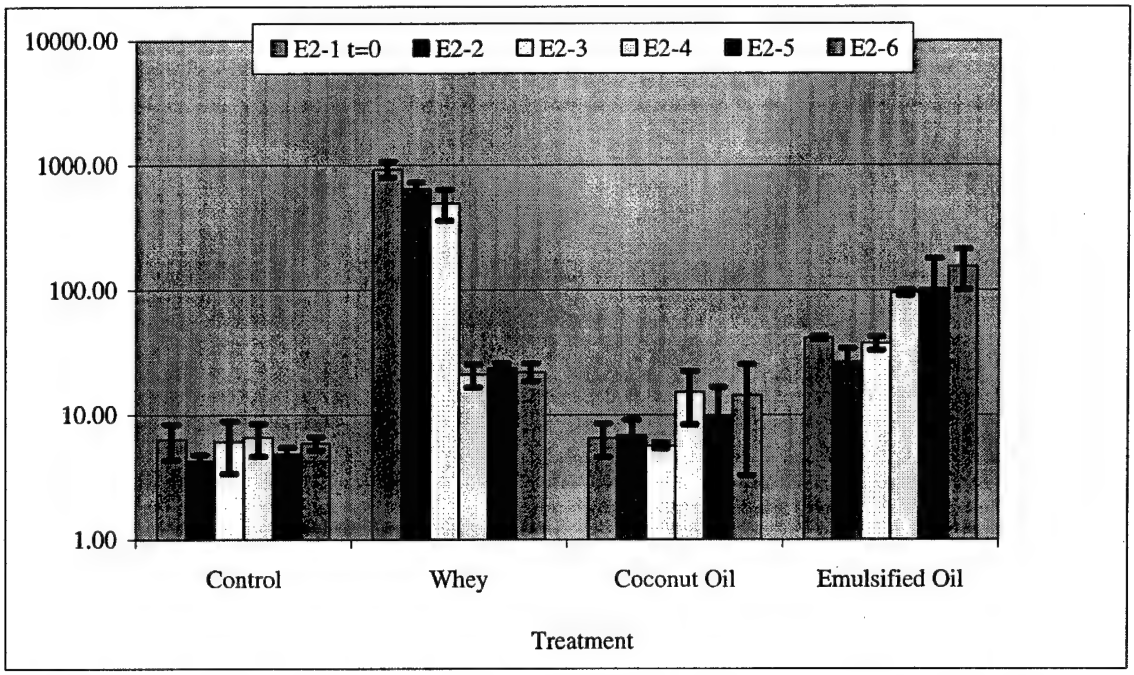


Figure 6.13. Mean DOC concentrations measured in biotic reactors as a function of sampling time and carbon donor for non-bioaugmented microcosms. Note: Y-axis is shown as a logarithmic scale.

dissolved carbon over time as the reservoir of insoluble carbon was hydrolyzed over time. This oil metabolism and hydrolysis was more prominent, however, in the Emulsified Oil treatment, where DOC steadily rose from initial levels of approximately 40 mg/L, to over 200 mg/L by then end of the 85-day incubation period for this treatment.

With the creation of reduced conditions in response to the addition of carbon donor, came not only the excessive levels of dissolved iron (> 80 mg/L) in solution as shown in Figure 6.11, but also elevated levels of dissolved arsenic in the form of As^{3+} (Figure 6.14) and elevated headspace concentrations of methane (Figure 6.15). High dissolved iron, As^{3+}, and methane were particularly evident in the Whey amended reactors, where 40 to 70 µg/L dissolved arsenic persisted through most of the 280-day incubation period, and the mean reactor headspace methane concentration peaked at more than 35vol% during the study. These high methane levels indicate that carbon donor was shunted to methnogensis rather than to TCE degradation despite conditions that appear generally favorable for dechlorination in the microcosms receiving carbon addition. Dissolved arsenic concentrations in the other carbon donor augmented systems approached the current drinking water standard of 50 µg/L, and greatly exceeded the proposed 10 µg/L standard for most of the study. While elevated iron levels appeared transitory in the Whey amended reactors, dissolved arsenic persisted unacceptably high levels in all treatments, making carbon donor addition alone, particularly without the subsequent stimulation of TCE dechlorination, an ineffective and possibly detrimental technology if applied at OU5.

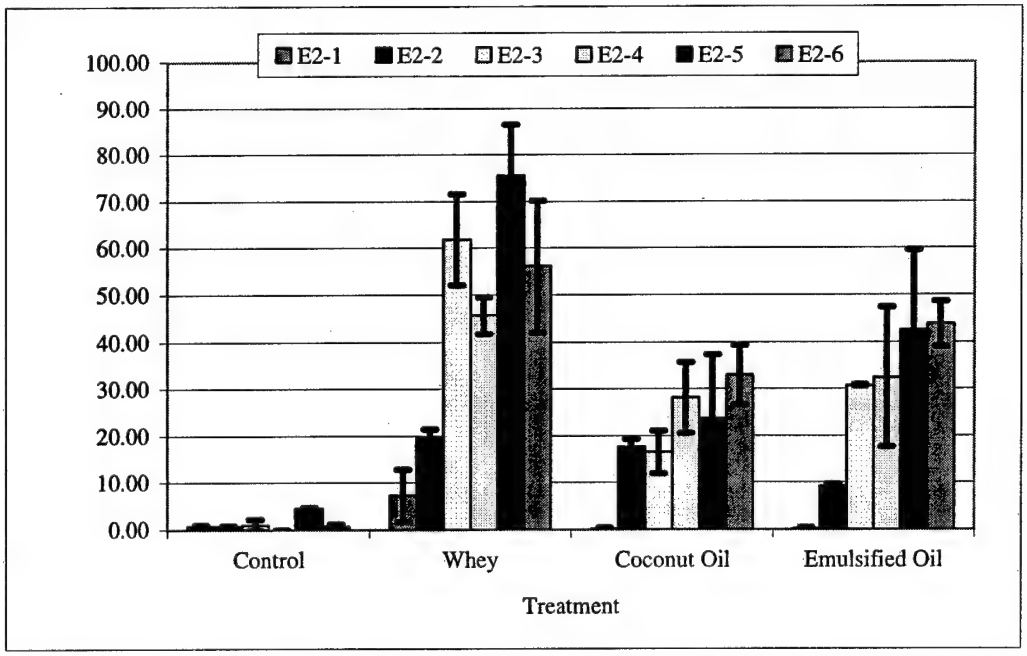


Figure 6.14. Mean As^{3+} concentrations measured in biotic reactors as a function of sampling time and carbon donor for non-bioaugmented microcosms.

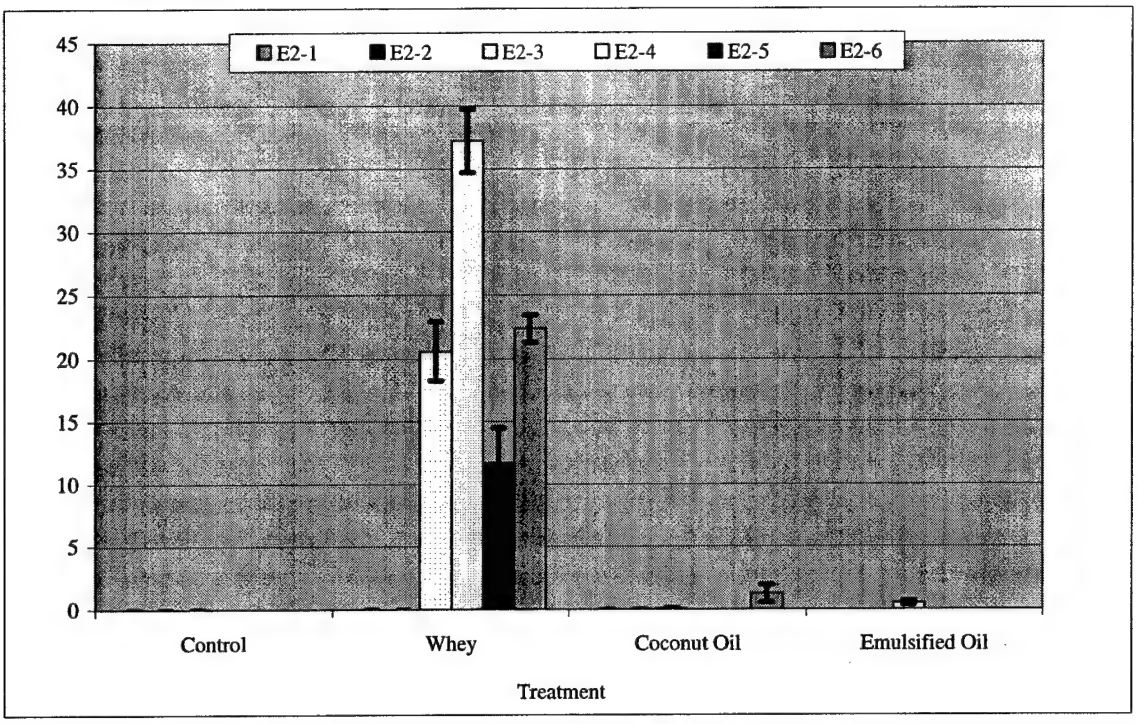


Figure 6.15. Mean CH_4 concentrations measured in biotic reactor headspace as a function of sampling time and carbon donor for non-bioaugmented microcosms.

Concentration profiles for dissolved iron, sulfate, and DOC over time in the unamended, Control Reactors during the 280-day incubation period are shown in Figure 6.16. These data show little change in any of the analytes and represent a non-reactive control as intended. In contrast, Figures 6.17 to 6.19 reflect the trends in these analytes observed in carbon donor amended treatments. Figure 6.17 shows the data from the Whey amended reactors over a 280-day incubation period, indicating both a significant reduction in added carbon donor supply (≈ two orders of magnitude), the production of iron (maximum four order of magnitude change) and dissolved arsenic (≈ one order of magnitude), and the utilization of sulfate (≈ two order of magnitude reduction) in response to this carbon donor utilization.

Figure 6.18 shows the response of dissolved iron, sulfate, DOC, and arsenic over time in the Coconut Oil treatment. Here, no significant DOC changes were observed during incubation as the residual oil in the reactors slowly solubilized. Both dissolved iron and arsenic did increase by approximately two orders of magnitude, however, and sulfate decreased by approximately two orders of magnitude during incubation clearly demonstrating significant microbial activity taking place in these reactors over time. Microcosms amended with Emulsified Oil (Figure 19) responded in a manner similar to the Coconut Oil treatments, but the response was larger, resulting in a four order of magnitude decrease in sulfate concentrations, and an approximate three order of magnitude increase in dissolved iron and arsenic over time. Again, biological activity is clearly taking place in the carbon amended systems, but this activity is not leading to TCE dechlorination in the OU5 aquifer material.

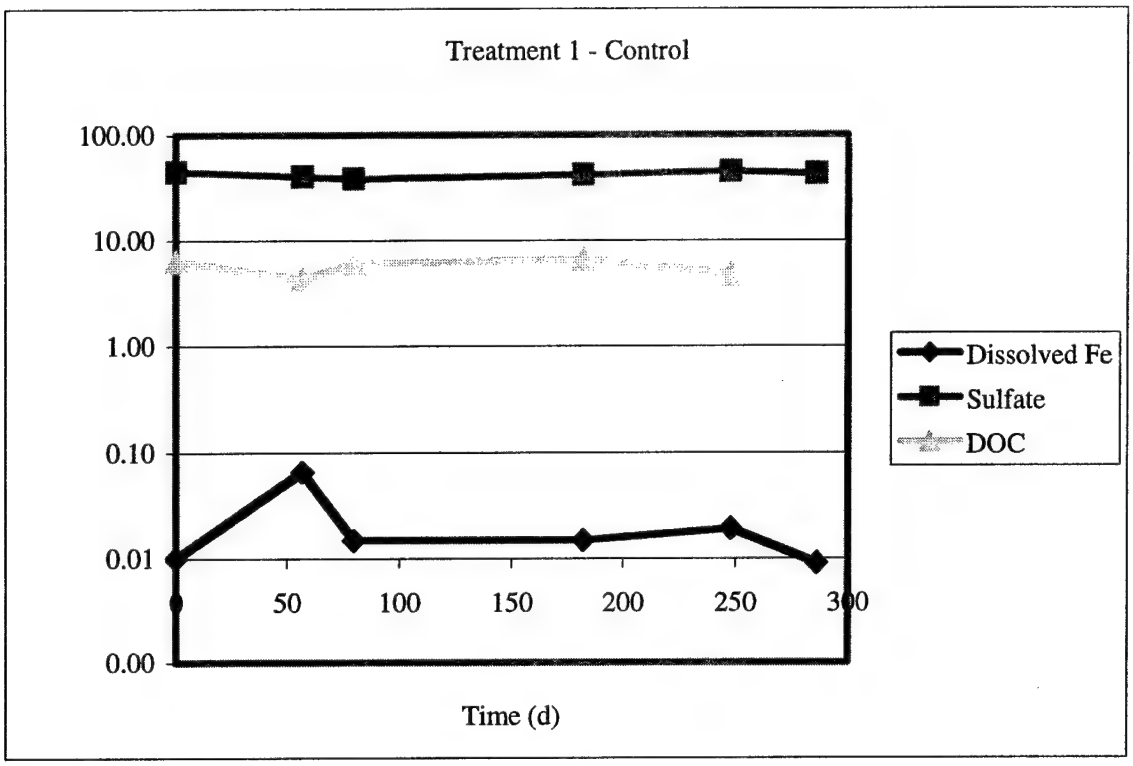


Figure 6.16 Dissolved iron, sulfate, and DOC concentrations in biotic microcosm reactors in Control systems (without donor, without microbial amendment) over time. Note Y-axis shown as a log scale.

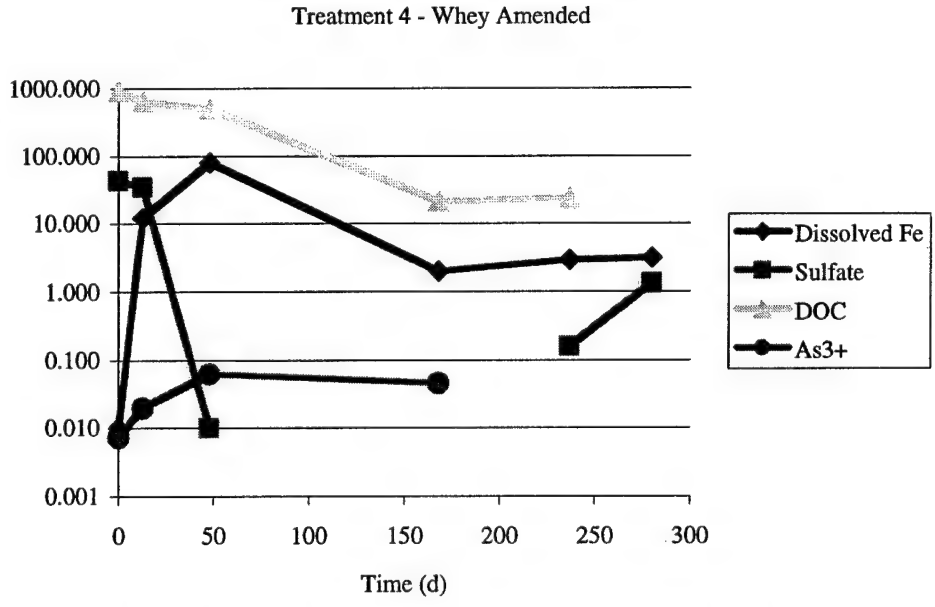


Figure 6.17 Dissolved iron, sulfate, and DOC concentrations in biotic microcosm reactors in Whey amended systems (without microbial amendment) over time. Note Y-axis shown as a log scale.

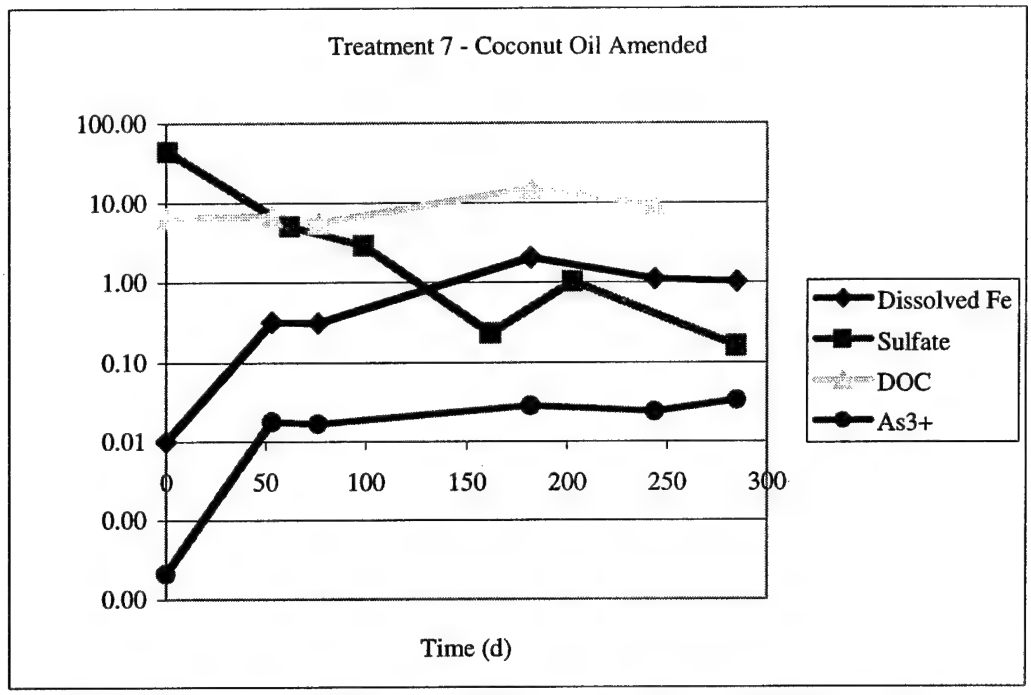


Figure 6.18 Dissolved iron, sulfate, and DOC concentrations in biotic microcosm reactors in Coconut Oil amended systems (without microbial amendment) over time. Note Y-axis shown as a log scale.

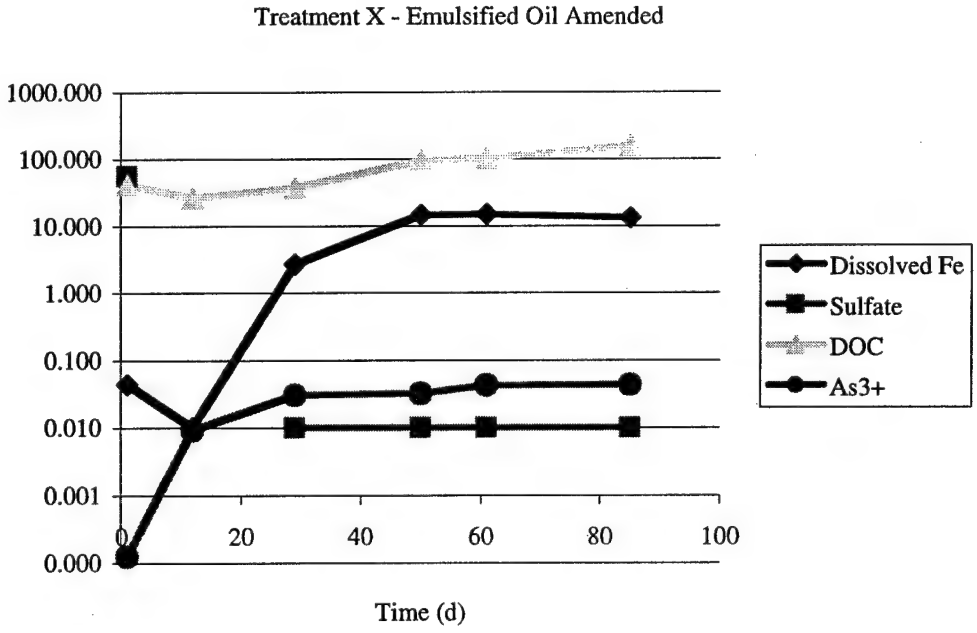


Figure 6.19 Dissolved iron, sulfate, and DOC concentrations in biotic microcosm reactors in Emulsified Oil amended systems (without microbial amendment) over time. Note Y-axis shown as a log scale.

6.3.2. Degradation results from bioaugmented reactors. This bioaugmentation study included: a Control Reactor; the Bachman Road Culture amended with Whey, Coconut Oil and Emulsified Oil; and a ZVI abiotic control treatment. Final TCE, cis-DCE, and VC molar concentration data in the biotic reactors over the complete 70 to 83 day incubation period are summarized graphically in Figure 6.20, 6.21, and 6.22, respectively. All raw chlorinated hydrocarbon data for this experiment are included in Appendix P.

Three distinct sets of reactors are evident from the time course of TCE concentrations shown in Figure 6.20. The first set of reactors represent controls of various types, and include the Soil Control (TCE with no carbon donor or innocula amendment), and the MBI and BR Controls (TCE with microbial innocula but no carbon donor). These control treatments showed the smallest change in TCE concentration over time, and have the highest residual TCE concentration at the end of the study as expected. The next set of reactors are those which produce a pseudo first-order decrease in TCE concentration throughout the entire incubation period. This group of reactors includes: BR + Whey, BR + Coconut Oil, MBI + Coconut Oil, and the ZVI treatments. These treatments generally supported complete TCE dechlorination by the end of the 80-day study period. The final set of reactors produced rapid and complete TCE dechlorination after a lag period of from 20 to 30 days, while reducing conditions developed within the reactors. This final set included: the MBI + Whey, the BR + Emulsified Oil, and the MBI + Emulsified Oil treatments.

TCE transformation products in the form of cis-DCE (Figure 6.21) were prominent features of the MBI amended treatments regardless of carbon used, while only small levels of cis-DCE accumulated in the BR amended treatments. VC (Figure 6.22) was the major degradation product in the BR + Emulsified Oil system, although it was transformed completely by the end of the study. VC tended to be more persistent and more slowly produced in the MBI augmented systems than when BR was used as the microbial amendment. No significant levels of either cis-DCE or VC were produced in the ZVI treatment reactors as is expected from this abiotic chemistry.

TCE transformation in the microcosms amended with the Bachman Road culture and various carbon donor sources (Whey, Coconut Oil, and Emulsified Oil) are shown in Figures 6.23, 6.24, and 6.25, respectively. Neither the Whey nor the Coconut Oil amendments produced TCE degradation rates significantly greater than in the Abiotic Control reactors, but both did shown evidence of the stimulation of TCE degradation based on changes observed for cis-DCE and VC during incubation. VC concentrations were observed to accumulate slightly in the Bachman Road + Whey reactors beginning approximately 50 days of incubation (Figure 6.23) following the increase in TCE loss in these reactors that began on Day 40 of incubation. Both cis-DCE and VC were found to be slightly increasing in the Bachman Road + Coconut Oil treatment from Day 15 of incubation, and continued to increase through the end of the study. TCE and daughter product transformation within the Bachman Road + Emulsified Oil reactors was significantly different as shown in Figure 25. In this treatment, cis-DCE and VC rapidly increased, and TCE concentrations rapidly decreased after 10 to 20 days of incubation.

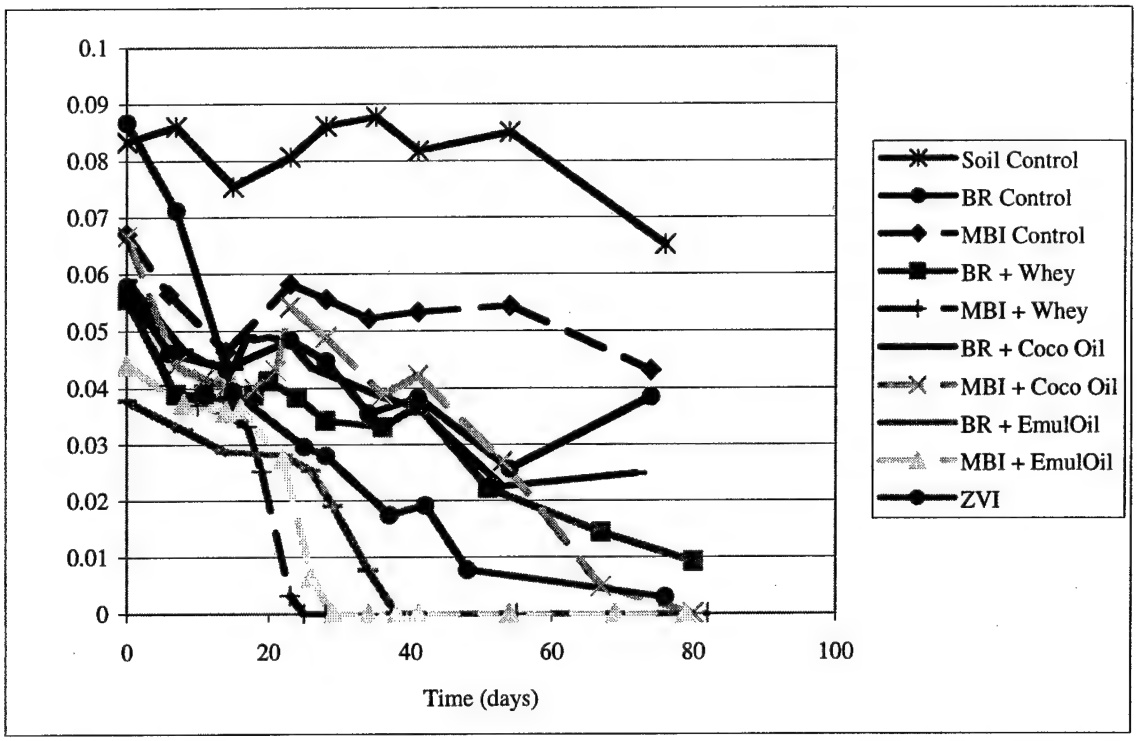


Figure 6.20. Variation of TCE concentrations over time in the bioaugmented microcosms as a function of carbon donor amendment.

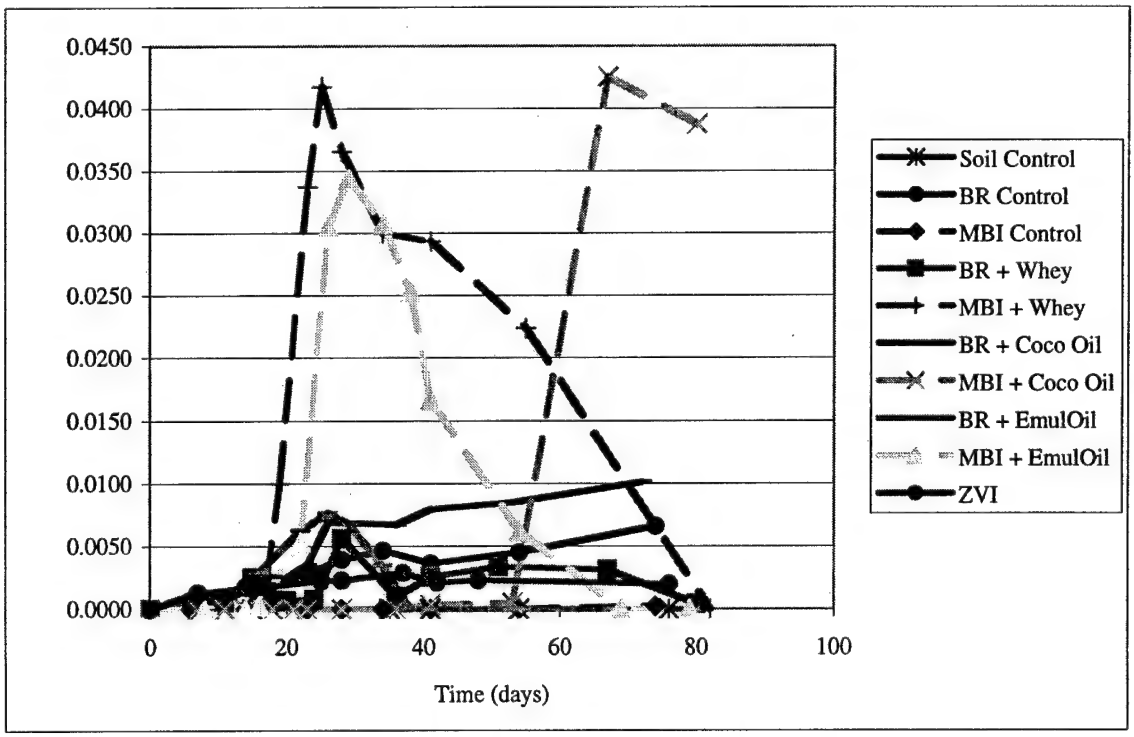


Figure 6.21. Variation of cis-DCE concentrations over time in the bioaugmented microcosms as a function of carbon donor amendment.

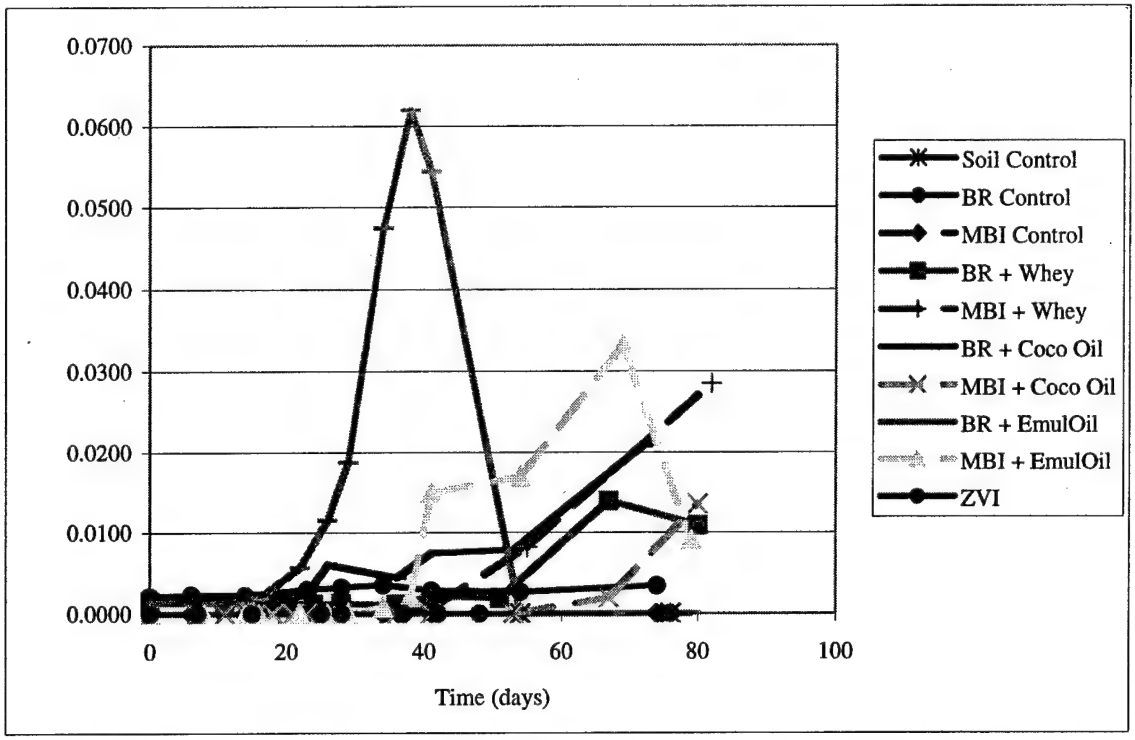


Figure 6.22. Variation of VC concentrations over time in the bioaugmented microcosms as a function of carbon donor amendment.

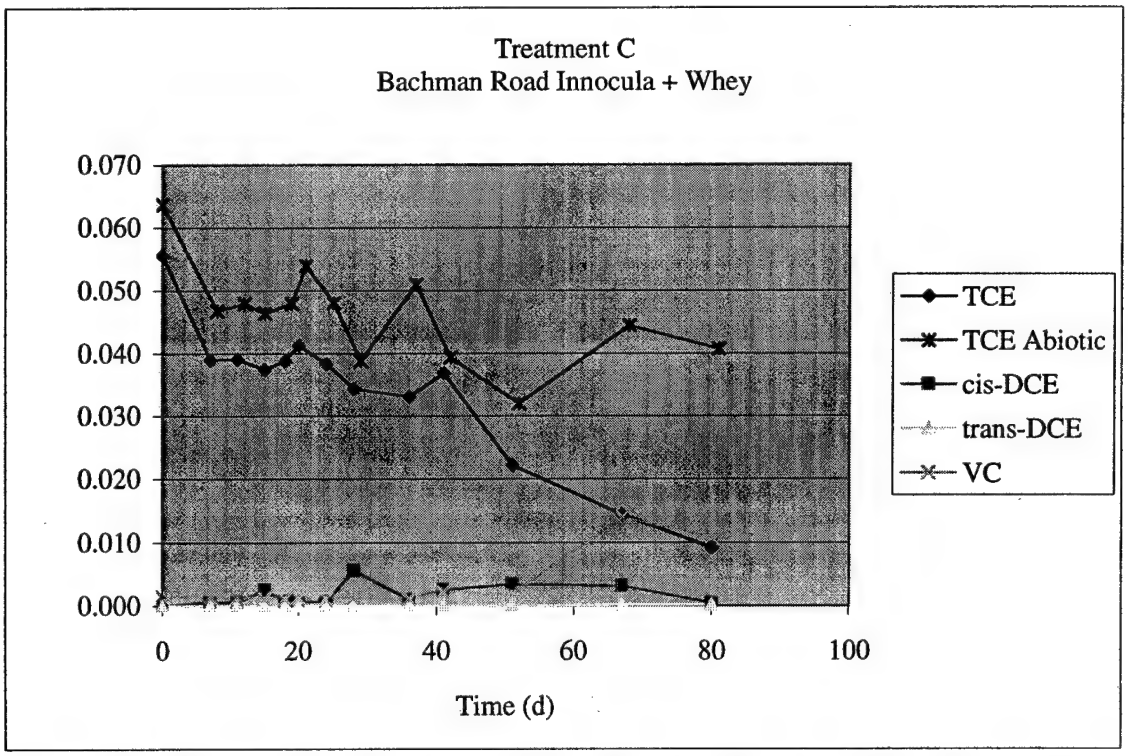


Figure 6.23. The time variation in the Bachman Road + Whey Treatment reactors over an 80-day incubation period. Note concentrations represented as mmol/L.

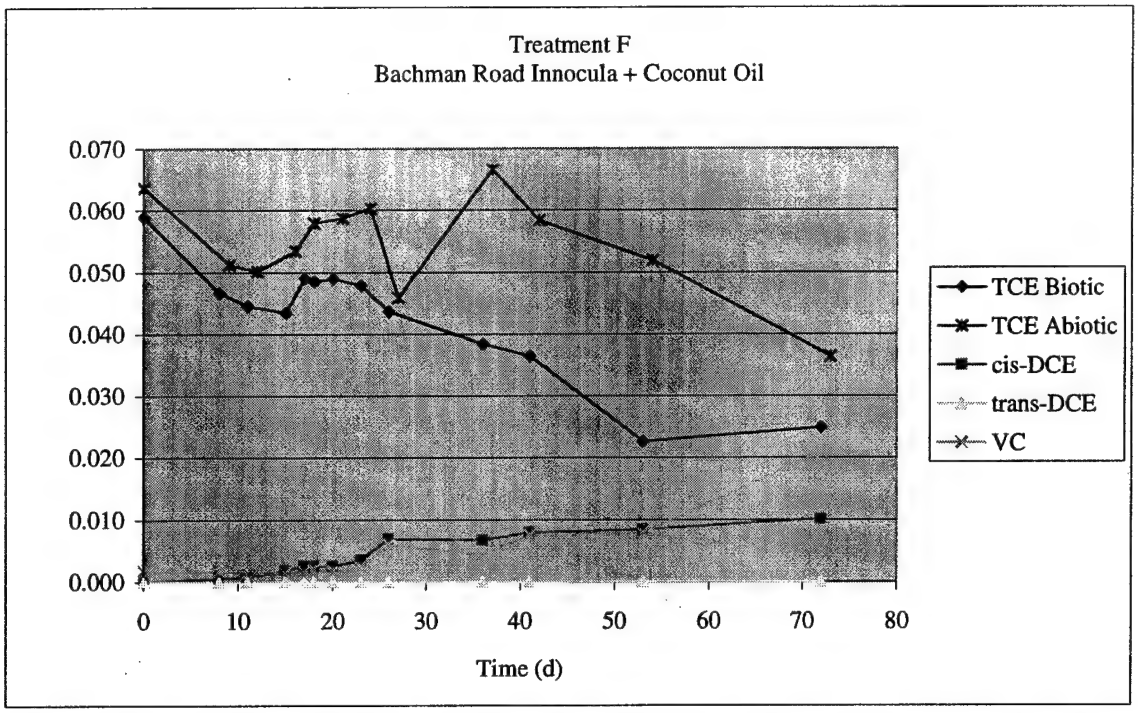


Figure 6.24. The time variation in the Bachman Road + Coconut Oil Treatment reactors over a 75-day incubation period. Note concentrations represented as mmol/L.

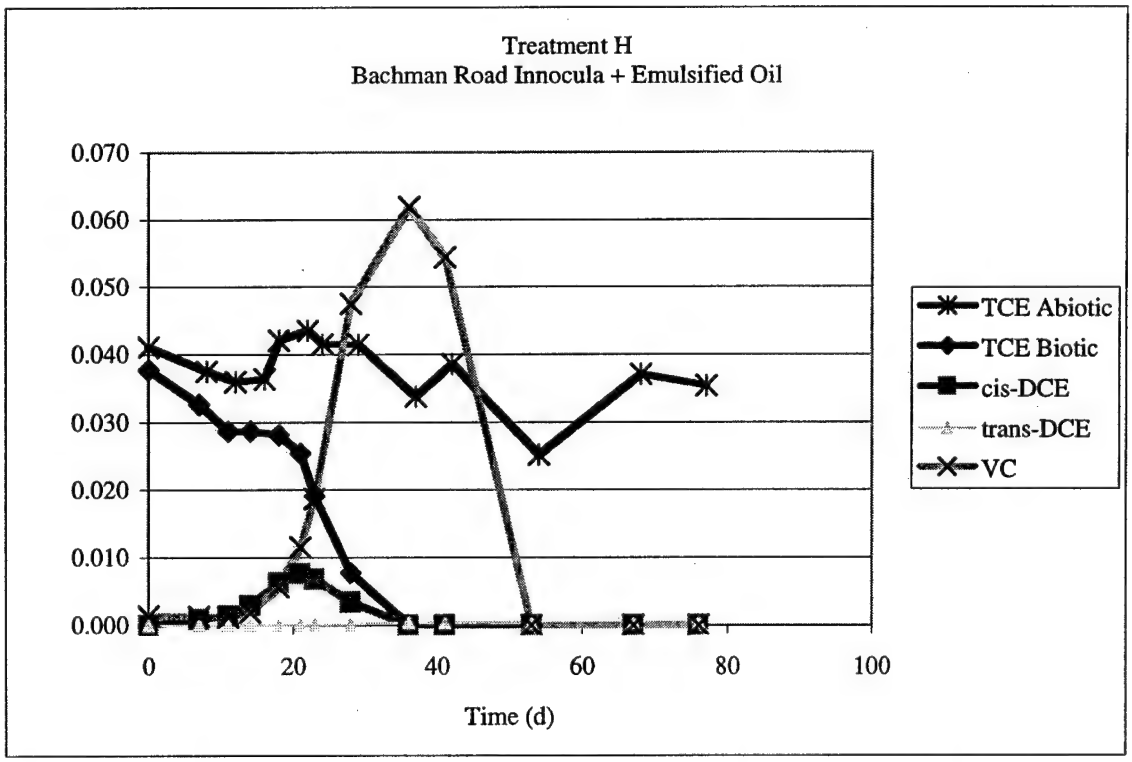


Figure 6.25. The time variation in the Bachman Road + Emulsified Oil Treatment reactors over a 78-day incubation period. Note concentrations represented as mmol/L.

The accumulation of cis-DCE was minimal as it peaked on Day 20 at less than 0.01 mmol/L before falling to non-detect levels by Day 35 of incubation. TCE transformation was also complete by Day 35. VC accumulated to very high levels (0.060 mmol/L or 3.87 mg/L) between Day 10 and Day 40, prior to falling to non-detect levels by Day 50 of incubation.

TCE transformation relationships for the MBI Granular Culture plus various carbon donor amendments were different than those observed for the Bachman Road culture as shown in Figures 6.26, 6.27 and 6.28. With the MBI Granular Culture, all carbon donors produced a rapid reduction in TCE concentration after 2 to 5 weeks incubation that resulted in various levels of intermediate product accumulation in the microcosm reactors over time. The MBI Granule + Whey reactors (Figure 6.26) showed a rapid TCE transformation to non-detect levels between 18 and 22 days of incubation, coupled with a rapid increase in cis-DCE over the same period. Beyond Day 22, cis-DCE was slowly transformed to VC, so that by the end of the 82-day incubation study, only VC remained in these reactors. TCE transformation in the MBI Granule + Coconut Oil treatment was much slower than with Whey, requiring approximately 50 days for the initiation of cis-DCE production in the microcosms. cis-DCE levels peaked by the next sampling event on Day 70, with cis-DCE and minor concentrations of VC remaining within the reactors at the end of the study on Day 80. The most rapid transformation of TCE and its daughter products with the MBI Granular amendment was in the Emulsified Oil carbon donor reactors as shown in Figure 6.28. Here, as with the Bachman Road + Emulsified Oil treatment, TCE transformation to cis-DCE was rapid and complete, but unlike the Bachman Road amended microcosms, the MBI Granular culture accumulated cis-DCE before VC production was initiated. VC accumulation continued to Day 70 as cis-DCE was degraded. VC degradation in the MBI Granule + Emulsified Oil reactors did not fall to non-detect levels as it did in the Bachman Road treatment, but was reduced by approximately 67% by the end of the 80-day incubation period.

Finally, chlorinated hydrocarbon concentration changes over time in the batch Zero Valent Iron treatment are shown in Figure 6.29. TCE transformation in this reactive, abiotic treatment was approximately first-order with respect to time, The reaction rate for the biotic and abiotic treatments were essentially identical, and the reaction did not generate significant levels of DCE or VC intermediate products over the course of the study as is expected from this abiotic reaction process.

Transformation rates from the microbial amendment plus carbon donor treatments are qualitatively summarized in Table 6.4. Quantitative TCE and daughter product transformation rates were estimated using the procedures defined above, i.e., by: 1) converting all mass concentration data to molar concentrations, 2) normalizing these concentrations measured over time (C) to the initial concentrations measured in the reactors at time 0 (Co), and 3) taking the natural log transformation of these normalized concentrations to generate values of ln(C/Co) at each sampling interval. These log-transformed concentration values were then regressed against corresponding sampling times in days to generate an estimate of a first-order loss rate for each treatment (the slope of this linear regression relationship). Degradation rates were then estimated by

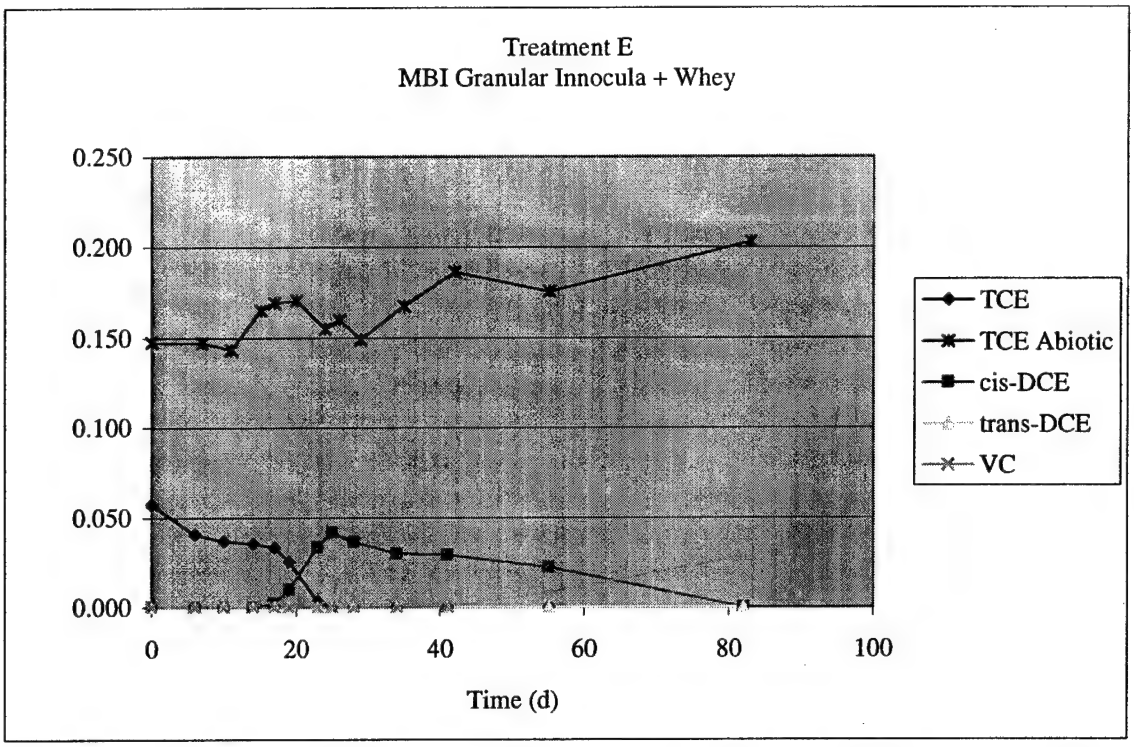


Figure 6.26. The time variation in the MBI Granule + Whey Treatment reactors over an 82-day incubation period. Note concentrations represented as mmol/L.

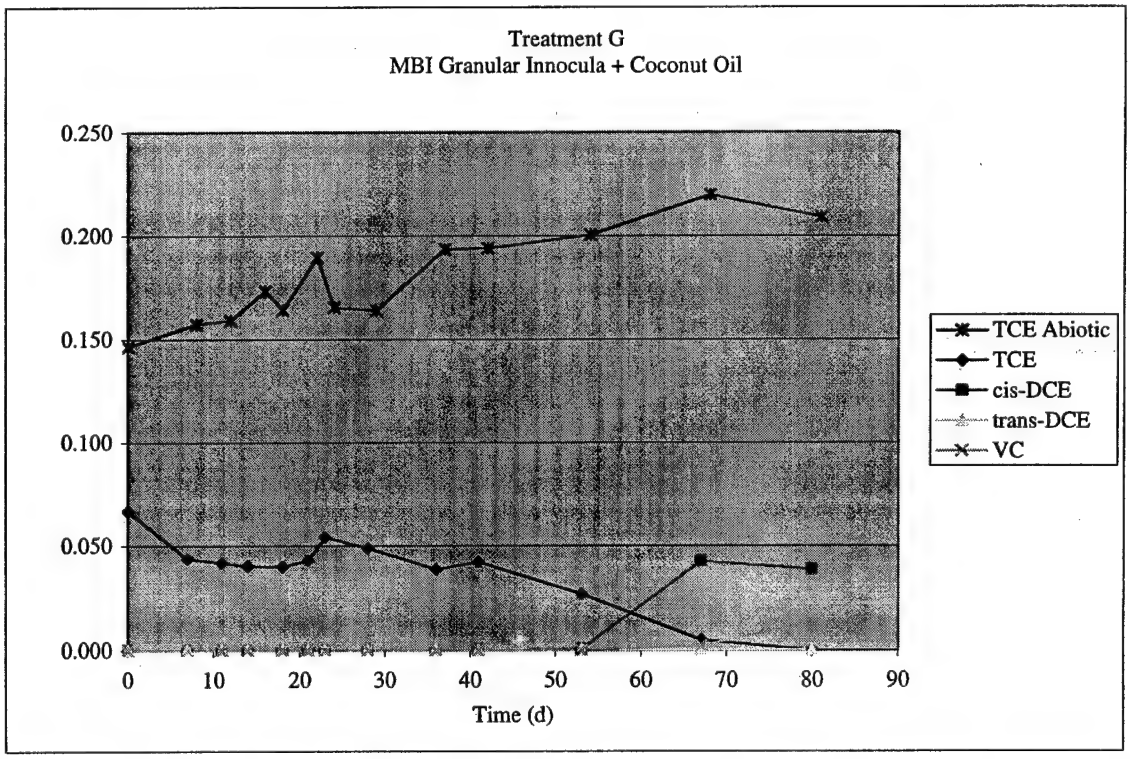


Figure 6.27. The time variation in the MBI Granule + Coconut Oil Treatment reactors over an 80-day incubation period. Note concentrations represented as mmol/L.

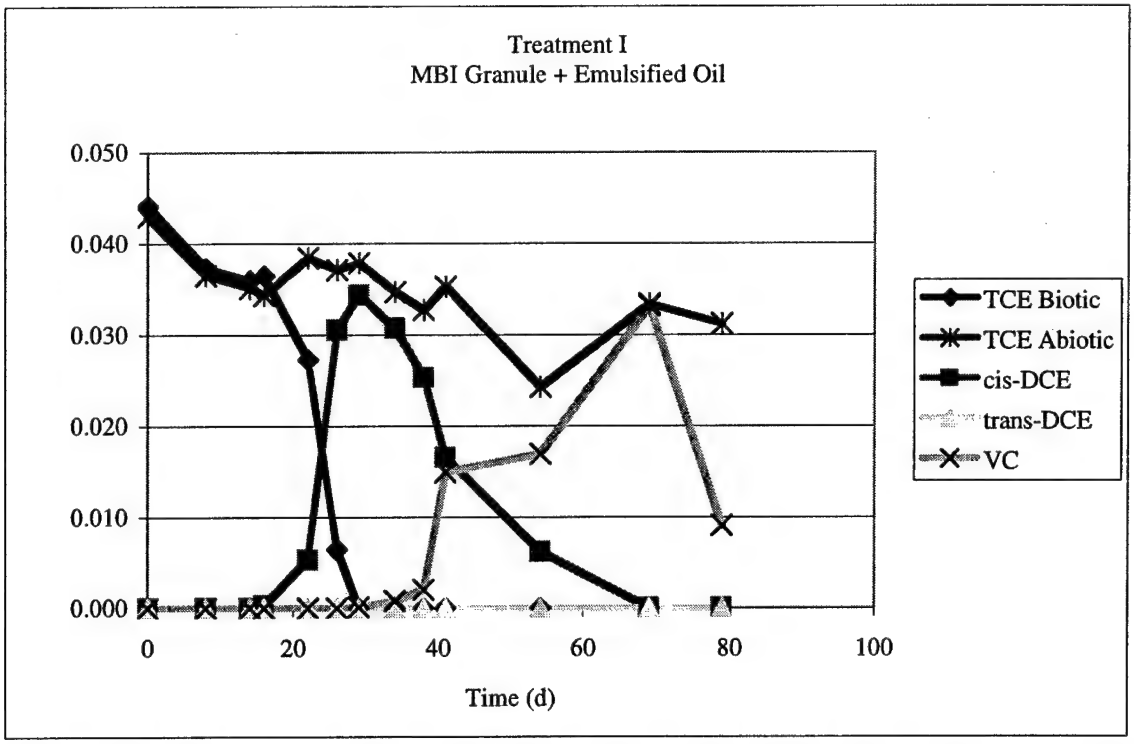


Figure 6.28. The time variation in the MBI Granule + Emulsified Oil Treatment reactors over a 79-day incubation period. Note concentrations represented as mmol/L.

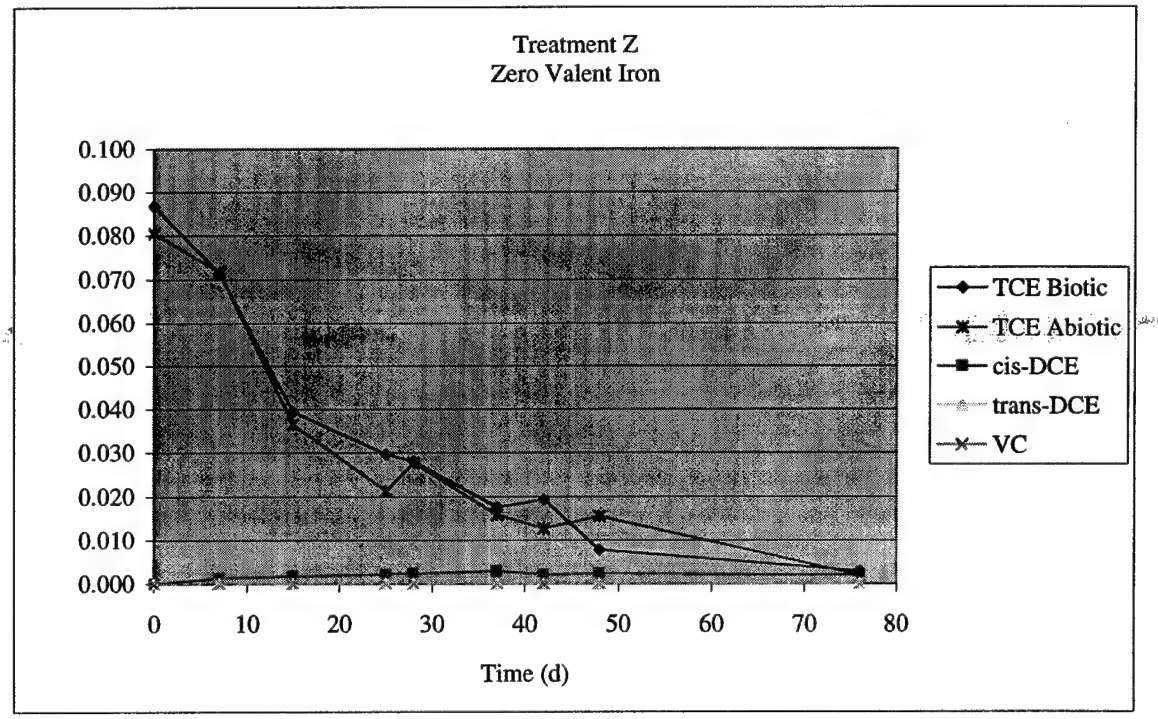


Figure 6.29. The time variation in the ZVI Treatment reactors over a 78-day incubation period. Note concentrations represented as mmol/L.

Table 6.4. Qualitative TCE and daughter product transformation rates for the microbial innocula plus carbon donor reactors.

Treatment	Time to Complete TCE Transformation	Time to Complete cis-DCE Transformation	Time to Complete VC Transformation
A – Soil Control	>> 80 days	No Product	No Product
B – Bachman Road Control	>> 80 days	>>> 80 days	>>> 80 days
C – Bachman Road + Whey	> 80 days	> 80 days	>> 80 days
F – Bachman Road + Coconut Oil	> 80 days	>> 80 days	>> 80 days
H – Bachman Road + Emulsified Oil	36 days	36 days	53 days
D – MBI Granule Control	>> 80 days	>>> 80 days	No Product
E-MBI Granule + Whey	25 days	82 days	>> 80 days
G – MBI Granule + Coconut Oil	80 days	> 80 days	>> 80 days
I – MBI Granule + Emulsified Oil	29 days	69 days	> 80 days
Z – ZVI Control	> 80 days	> 80 days	No Product

correcting each first-order loss rate for the loss rate observed in the Control treatment (abiotic reactors with carbon donor + microbial innocula) by subtraction of the Control first-order loss rate from the loss rate for each biotic carbon donor + microbial innocula treatment. An example of typical regression results are shown in Figure 6.30 for the MBI Granule + Emulsified Oil treatment, while the results of all calculations are summarized in Table 6.5. As indicated in Table 6.5, TCE transformation rates in the OU5 microcosms that were amended with the microbial innocula are one to two orders of magnitude higher than comparable carbon donor only treatments (Table 6.3), making microbial inoculation at this site a technically feasible and viable alternative that warrants further consideration.

Water quality data were also collected from these bioaugmented reactors over time to assess the impact of carbon donor and microbial amendment addition on water quality in the microcosms. Raw data for these general water quality parameters are located in Appendix Q. As indicated for the non-bioaugmented reactors, pH and conductivity are gross water quality indicators and could not generally be used to distinguish among treatments. The pH of the bioaugmented treatments ranged from 5.8 to 8.8, with a mean across all reactors of 7.9. As indicated in Figure 6.31, the pH was stable over the 30 to 60-day incubation represented by these data. EC (Figure 6.32) ranged from 0.709 to 3.45 µS/cm, and was seen to increase during incubation in the Whey plus microbial amendment reactors, likely due to significant fatty acid production in these systems.

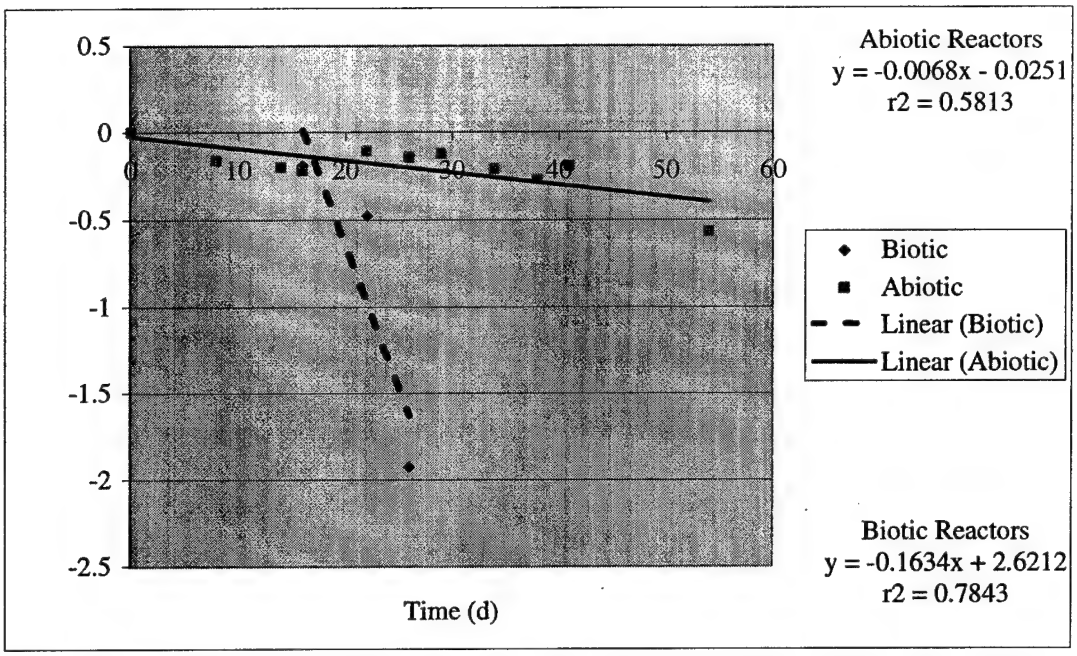


Figure 6.30. Example regression data from the bioaugmentation study, MBI Granule + Emulsified Oil treatment.

Table 6.5. Summary of TCE loss and Control-corrected TCE degradation rates observed during incubation of OU5 microcosms in complete culture augmentation study.

Treatment	TCE Loss Rate, Abiotic Control, 1/day	TCE Loss Rate, Biotic Reactors, 1/day	Control Corrected TCE Degradation Rate, 1/d
A – Soil Control	-0.0021	-0.0021	0, No Degradation
B – Bachman Road Control	-0.0038	-0.0069	-0.0031
C – Bachman Road + Whey	-0.0043	-0.0343	-0.030
F – Bachman Road + Coconut Oil	-0.0038	-0.0141	-0.0103
H – Bachman Road + Emulsified Oil	-0.0059	-0.339	-0.3331
D – MBI Granule Control	-0.0070	-0.0036	No Degradation
E-MBI Granule + Whey	-0.0073	-0.2694	-0.2621
G – MBI Granule + Coconut Oil	-0.0070	-0.0681	-0.0611
I – MBI Granule + Emulsified Oil	-0.0068	-0.1634	-0.1566
Z – ZVI Control	-0.0466	-0.0452	Abiotic Control

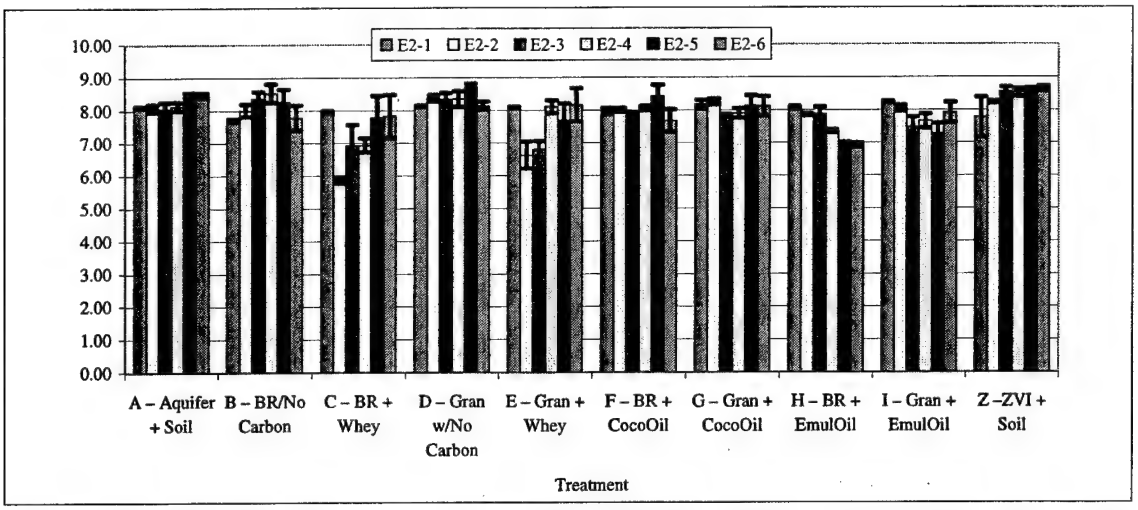


Figure 6.31. Variation of pH over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

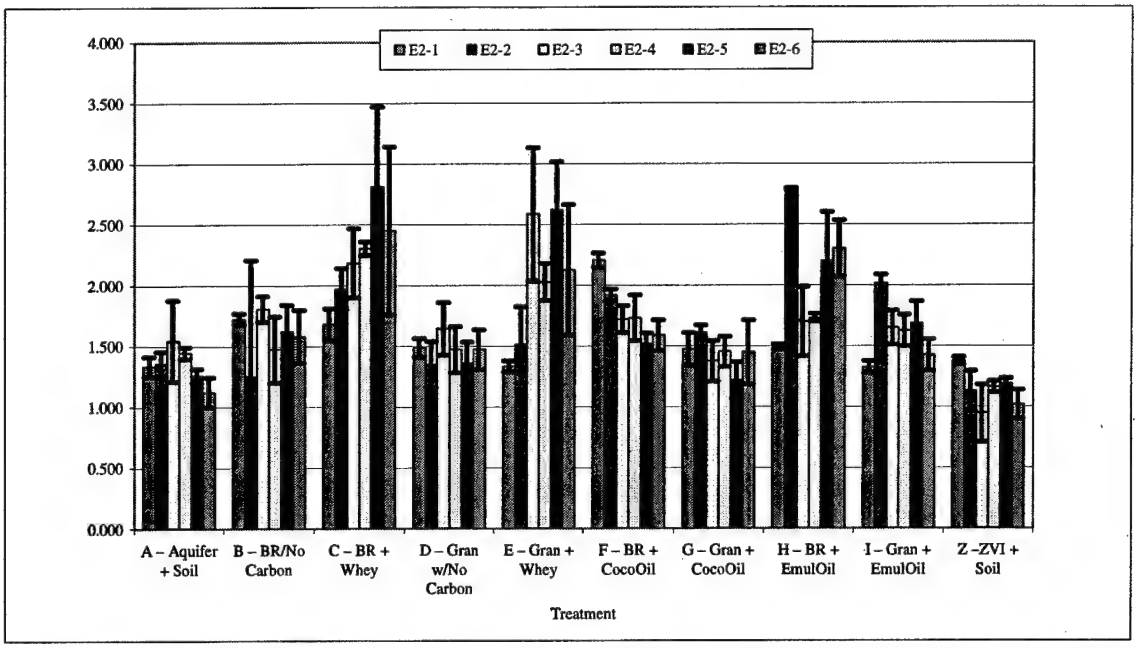
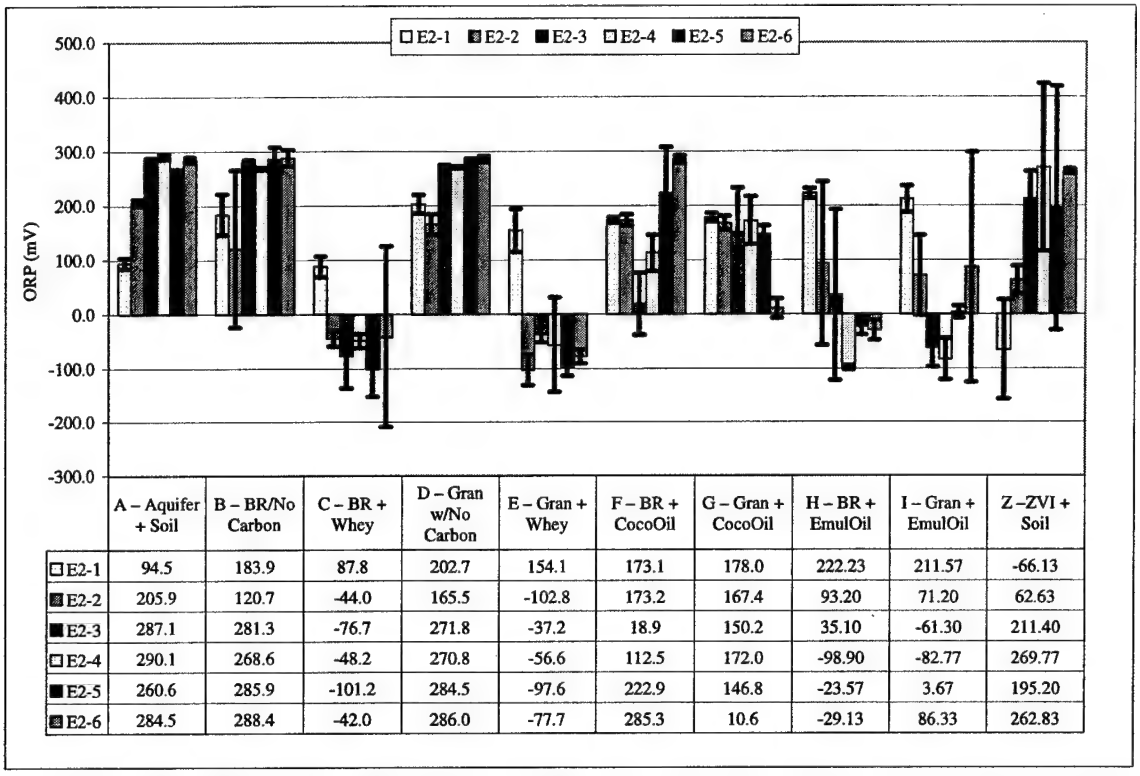


Figure 6.32. Variation of EC over time in complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

ORP was found to be much more variable than pH or conductivity as shown in Figure 6.33. The microbial amendment plus Whey and Emulsified Oil donor treatments displayed significantly decreased ORP values over the 30 to 60-day incubation period compared to initially measured values, and compared to the unamended treatments (Figure 6.10). Again, the Whey plus microbial amendment treatments showed the most consistent ORP, ranging from –40 mV to –100 mV beginning after only 7 days of incubation and remaining consistent through the end of the study.



	A – Aquifer + Soil	B – BR/No Carbon	C – BR + Whey	D – Gran w/No Carbon	E – Gran + Whey	F – BR + CocoOil	G – Gran + CocoOil	H – BR + EmulOil	I – Gran + EmulOil	Z –ZVI + Soil
E2-1	94.5	183.9	87.8	202.7	154.1	173.1	178.0	222.23	211.57	-66.13
E2-2	205.9	120.7	-44.0	165.5	-102.8	173.2	167.4	93.20	71.20	62.63
E2-3	287.1	281.3	-76.7	271.8	-37.2	18.9	150.2	35.10	-61.30	211.40
E2-4	290.1	268.6	-48.2	270.8	-56.6	112.5	172.0	-98.90	-82.77	269.77
E2-5	260.6	285.9	-101.2	284.5	-97.6	222.9	146.8	-23.57	3.67	195.20
E2-6	284.5	288.4	-42.0	286.0	-77.7	285.3	10.6	-29.13	86.33	262.83

Figure 6.33. Variation of ORP over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

Significant depletion of the electron acceptor pool was observed in all of the microcosm systems amended both with carbon donor and microbial innocula. Nitrate was essentially gone in all treatment reactors by Day 7 of incubation. Sulfate concentrations in Whey and Coconut Oil + microbial amendment treatments fell to 5 mg/L or less from initial levels of 40 to 60 mg/L within by 60 days of incubation, and in the Emulsified Oil only and Emulsified Oil + microbial amendment treatments within 10 days of incubation (Figure 6.34).

Significant iron reduction was also evident both in the Whey and Emulsified Oil treatments, where dissolved Fe^{2+} concentrations of 25 to 37 mg/L and approximately 15 to 35 mg/L were observed (Figure 6.35) at 60-days incubation, respectively. These peak iron concentrations were seen to decrease in all but the MBI Granule + Emulsified Oil treatment, so that maximum dissolved iron concentrations of 20 mg/L or less were measured by the end of the study period. Dissolved arsenic levels generally corresponded to the elevated iron concentrations observed in these reactors as shown in Figure 6.36, however, elevated As3+ concentrations were observed in all reactors receiving carbon donor amendment, not just those amended with Whey or Emulsified Oil. Arsenic concentrations measured at the end of the study increased in the microbially amended reactors in order of Emulsified Oil (25 to 35 µg/L), Coconut Oil (≈ 30 to 40

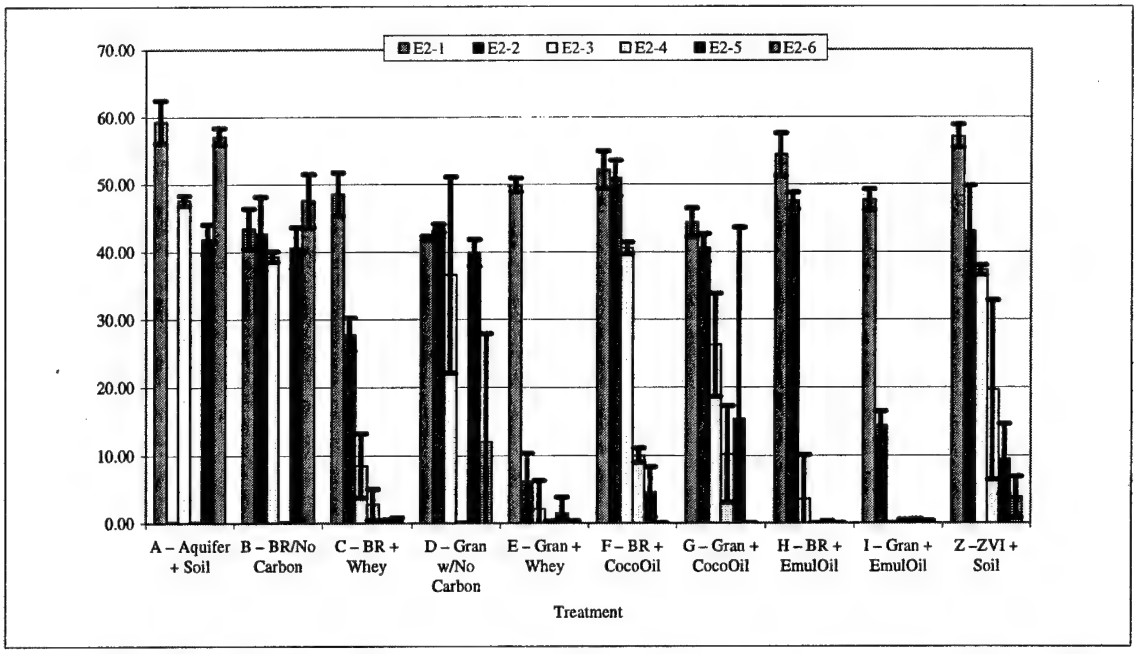


Figure 6.34. Variation of sulfate over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

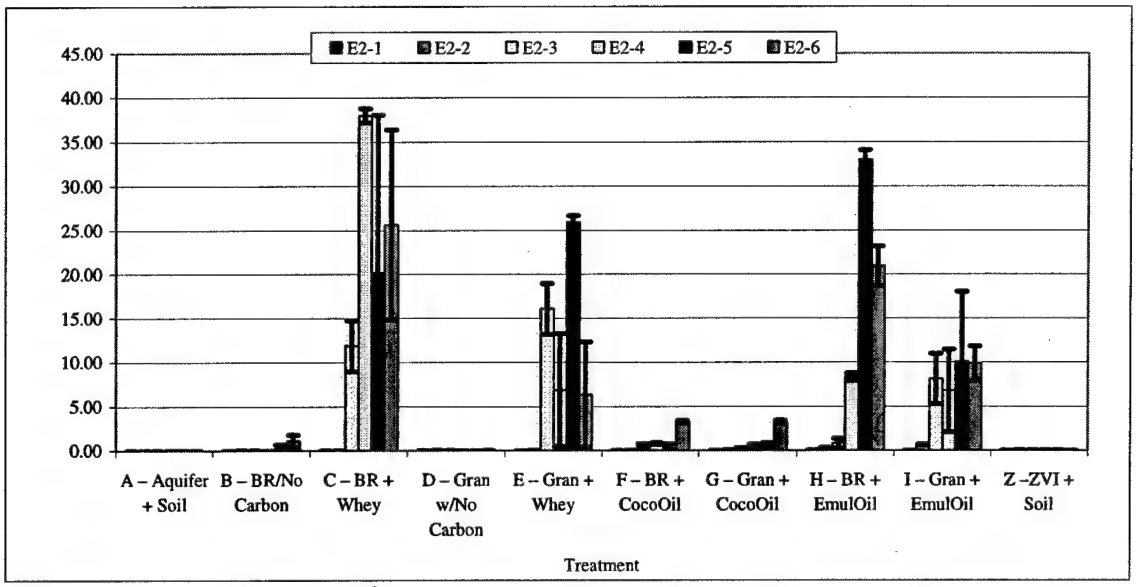


Figure 6.35. Variation of Fe^{2+} over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

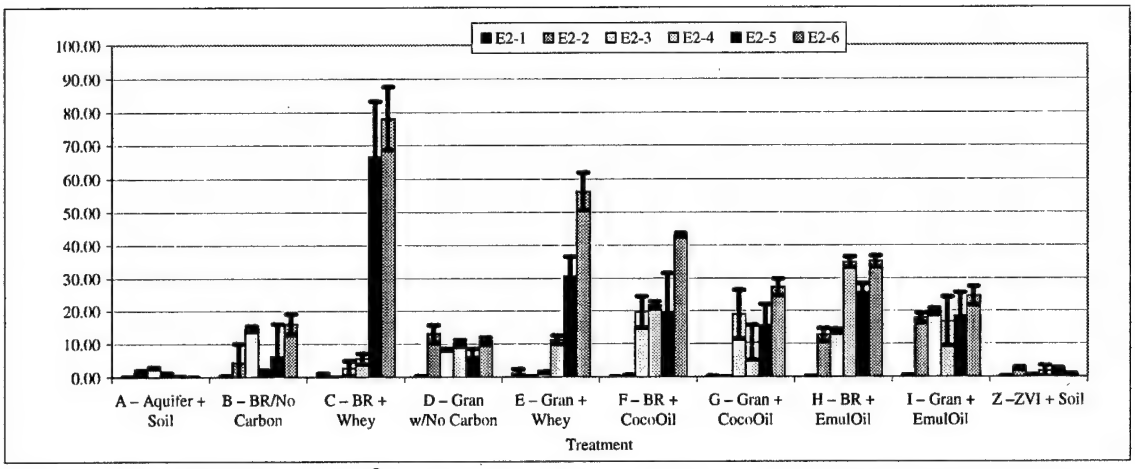
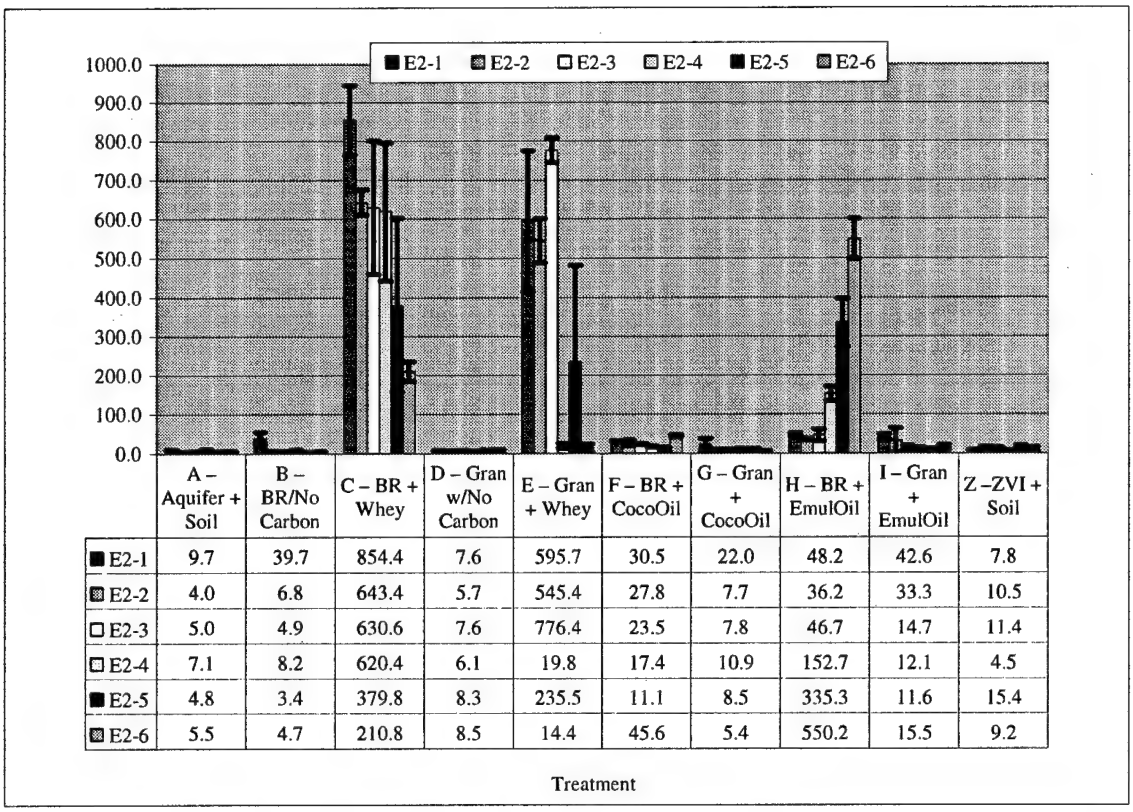


Figure 6.36. Variation of As^{3+} over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

µg/L), and Whey amendment (55 to 80 µg/L). These elevated concentrations were comparable to the maximum concentrations observed in the donor amendment only study, and would therefore be expected to be transitory over longer periods of incubation as was observed in the earlier study. Reprecipitation may occur more rapidly with microbial amendment as TCE transformation is complete within 80 days and the need for maintaining reducing conditions over the long-term with microbial innocula is eliminated. However, concerns over elevated arsenic concentrations in response to carbon donor and microbial amendment could limit the application of this technology at OU5, and arsenic dissolution and precipitation dynamics needs to be further investigated before full-scale implementation of this technology occurs.

Figure 6.37 presents DOC data available for all treatments through the end of the study. Several items are of note from these data. First, significant reductions in DOC concentrations were observed within the MBI Granule + Whey, and to a lesser extent, the Bachman Road + Whey treatment beginning 14 days after the start of incubation. This reduction in DOC within the MBI Granule + Whey treatment may be the cause of the low degradation rate of cis-DCE in these reactors. This effect was not seen, however, in the Bachman Road + Whey microcosms as this reduced carbon environment appeared at the time of the initiation of rapid TCE degradation and VC production. The other interesting observation is that in the Bachman Road + Emulsified Oil reactors, DOC concentrations were observed to increase by more than an order of magnitude beginning on Day 15 of incubation. This is consistent with the observation from the non-bioaugmented reactors for the Emulsified Oil only treatment, where its concentration increased by a factor of 4 during incubation. It is during this period that VC concentrations peaked then fell in the Bachman Road + Emulsified Oil treatment as shown in Figure 6.25. This increase in DOC due to degradation of the emulsified oil appears to have supported continual daughter product degradation, while resulting in only moderate levels of dissolved iron (Figure 6.35) and arsenic (Figure 6.36) release from the aquifer solids, making it the preferred carbon donor source of those evaluated in this study.



	A – Aquifer + Soil	B – BR/No Carbon	C – BR + Whey	D – Gran w/No Carbon	E – Gran + Whey	F – BR + CocoOil	G – Gran + CocoOil	H – BR + EmulOil	I – Gran + EmulOil	Z –ZVI + Soil
E2-1	9.7	39.7	854.4	7.6	595.7	30.5	22.0	48.2	42.6	7.8
E2-2	4.0	6.8	643.4	5.7	545.4	27.8	7.7	36.2	33.3	10.5
E2-3	5.0	4.9	630.6	7.6	776.4	23.5	7.8	46.7	14.7	11.4
E2-4	7.1	8.2	620.4	6.1	19.8	17.4	10.9	152.7	12.1	4.5
E2-5	4.8	3.4	379.8	8.3	235.5	11.1	8.5	335.3	11.6	15.4
E2-6	5.5	4.7	210.8	8.5	14.4	45.6	5.4	550.2	15.5	9.2

Figure 6.37. Variation of DOC over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

A final parameter of interest regarding the efficiency of carbon donor utilization during dechlorination is the level of methane within the bioaugmented microcosms. Methane headspace concentrations were periodically measured in the bioaugmented microcosms over the course of the study, and these data are summarized in Figure 6.38. As was observed in the non-bioaugmented reactors, methane generation was significant in the Whey amended treatments with both microbial innocula. Elevated levels of methane were also observed throughout the incubation period in the MBI Granule + Emulsified Oil treatment. Only minor levels of methane were measured in the Bachman Road + Emulsified Oil reactors until late in the incubation period when the pool of chlorinate hydrocarbons was depleted, suggesting that the Bachman Road culture is the preferred microbial amendment for potential application at OU5.

6.4 ZVI-TCE Degradation Study

6.4.1 Corrosion Products

Because of the rough surface of the iron corrosion samples, most of the useful information regarding corrosion products was contained in secondary, rather than backscatter electron images. As a result, only the secondary electron images are included

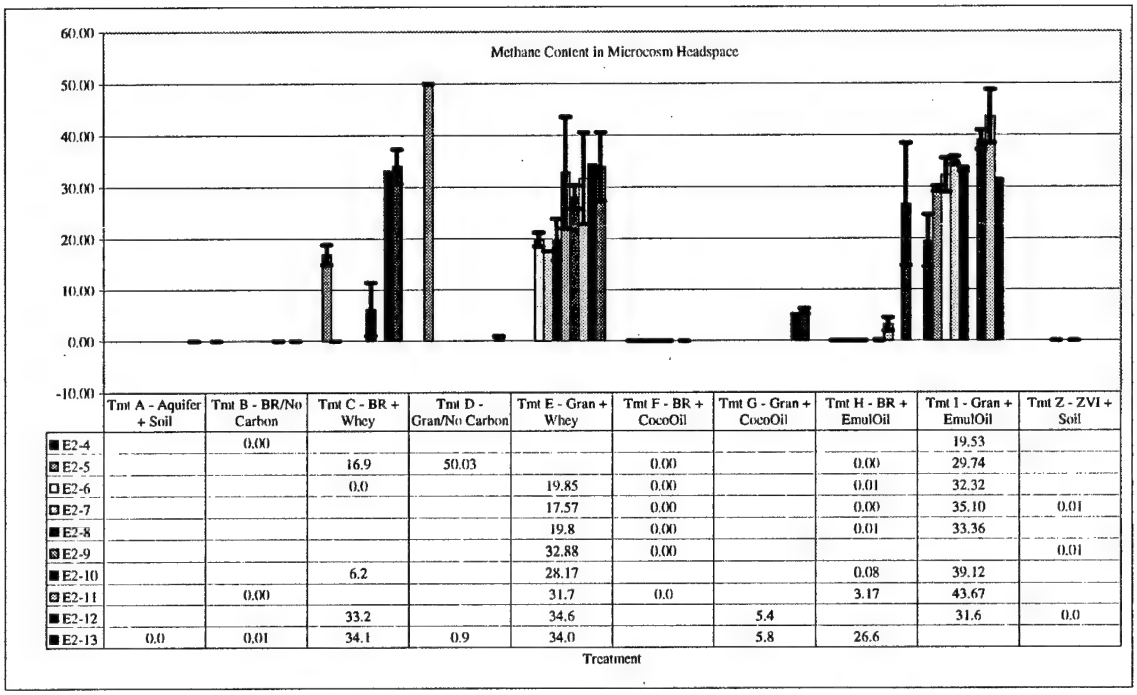


Figure 6.38. Variation of CH_4 concentrations measured in biotic reactor headspace over time in the complete culture augmentation study. Note bars represent 95% Confidence Intervals of the measurement.

here. Likely corrosion products formed under anaerobic conditions, considering the high carbonate content of groundwater at Hill AFB, are green rust and siderite. Under oxidizing conditions, Fe(III) oxides and carbonates would form. Although the forms of iron corrosion products that were present in these samples cannot be conclusively determined, different crystal structures in the corrosion product can be related to different minerals formed on the corroded iron (Table 6.6).

Table 6.6. Structure of crystal and possible corresponding iron corrosion product (Roh, et al., 2000 and Bigham, et al., 1989)

Structure	Iron Corrosion Products
Hexagonal crystal	Hematite, Akaganeite (β-FeOOH), Green rust
Cubic crystal	Maghemite (γ-Fe_2O_3)
Spherical Crystal	Ferrihydrite ($5Fe_2O_3.9H_2O$)
Acicular crystal	Goethite (α-FeOOH)

There were obvious differences in topography between the untreated Peerless ZVI (Figure 6.39) and the ZVI exposed to OU5 (OU12) groundwater with TCE over the 295

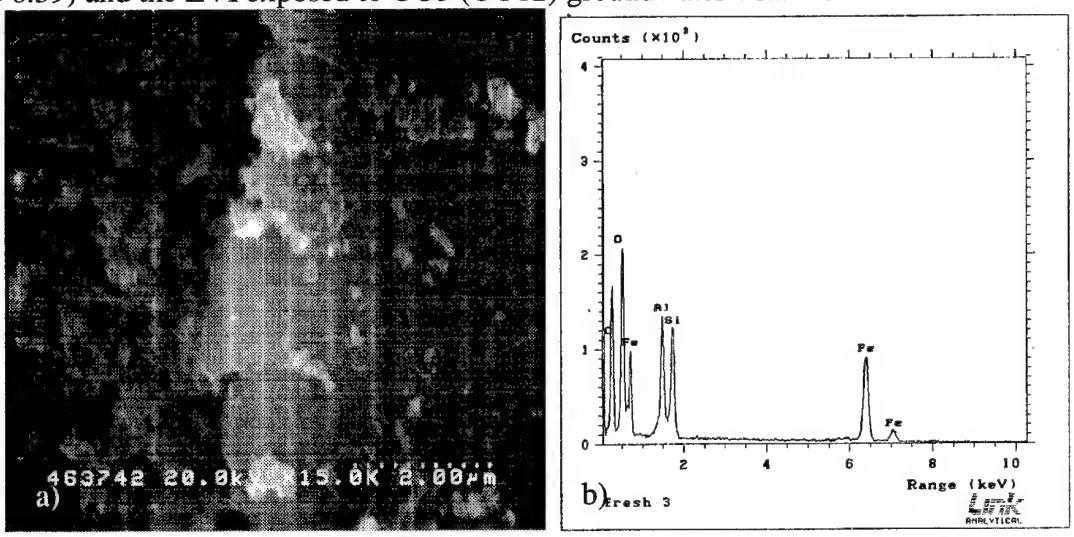


Figure 6.39. a) SEM image, and b) EDX spectrum of untreated Peerless iron.

-hour kinetic study. After this time period, the iron surface was largely covered with hexagonal, plate-like crystals (Figure 6.40), although some regions of what appeared to be uncorroded iron (Figure 6.41) and other crystal types (Figure 6.42) were observed. Hexagonal crystals composed mainly of iron indicated hematite, akaganeite, or green rust (Table 6.6).

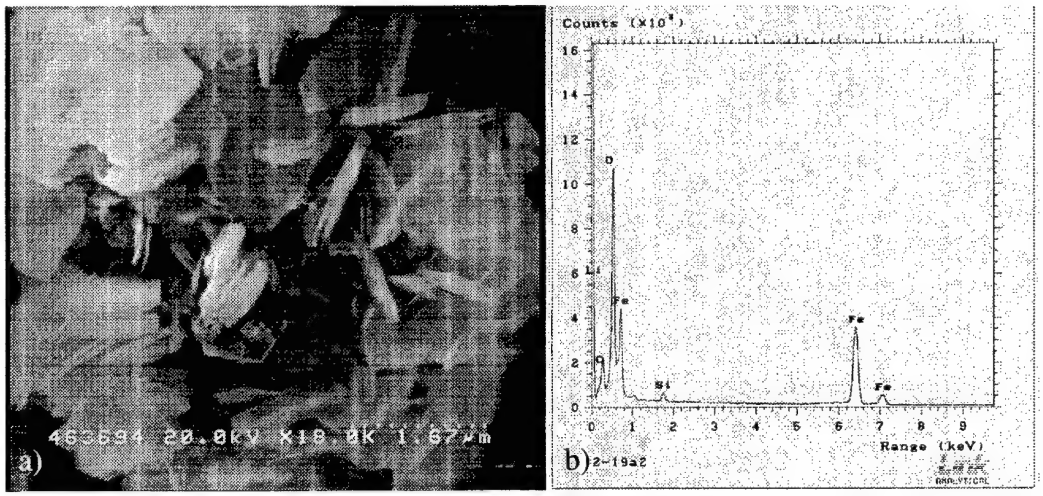


Figure 6.40. a) SEM image and b) EDX spectrum of Peerless iron, exposed to OU5 (OU12) TCE groundwater for 295 hours.

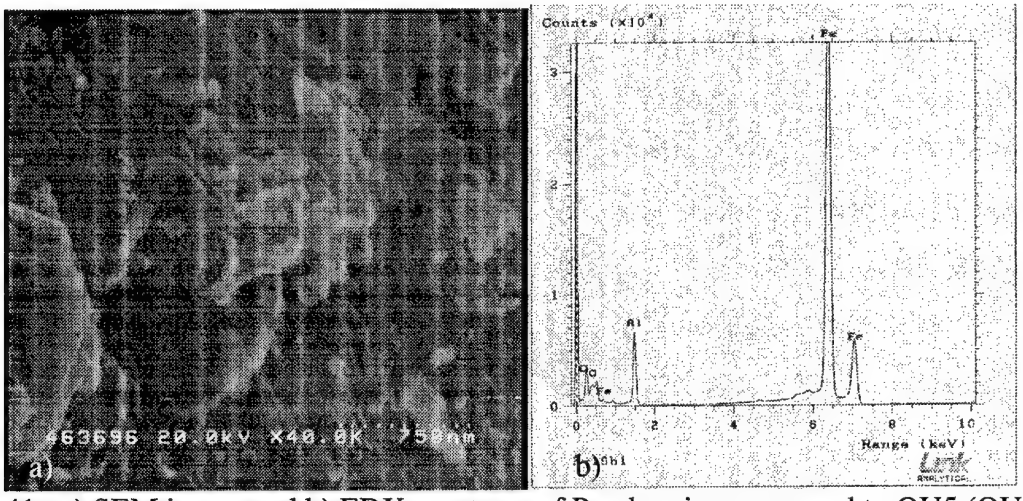


Figure 6.41. a) SEM image and b) EDX spectrum of Peerless iron, exposed to OU5 (OU12) TCE groundwater for 295 hours.

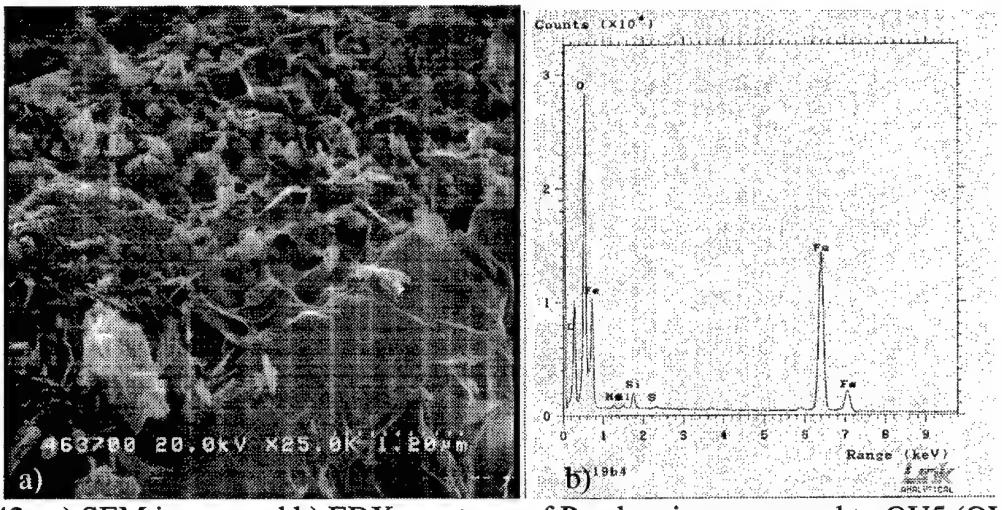


Figure 6.42. a) SEM image and b) EDX spectrum of Peerless iron, exposed to OU5 (OU12) TCE groundwater for 295 hours.

When Peerless iron was pre-corroded aerobically for 6 weeks, the ZVI was orange in color. SEM analysis confirmed the presence of amorphous iron oxide built-up on the surface (Figure 6.43), as well as the presence of a aluminum/silica phase (Figure 6.44). After the 96 hour TCE degradation study, hexagonal crystals were seen (Figure 6.45), as well as circular growths possibly indicating the presence of ferrihydrite (Figure 6.46) and an unidentified "spider web" type crystal (Figure 6.47).

Peerless iron was also pre-corroded anaerobically for 6 weeks, although no sample of this pre-corroded iron was analyzed on the SEM. There were, however, visual changes in the ZVI over this time period. After the 336 hour TCE degradation study, hexagonal crystals were seen (Figure 6.48 and 6.49) as well as the same unidentified "spider web" crystal (Figure 6.50) as was seen in the aerobically pre-corroded samples.

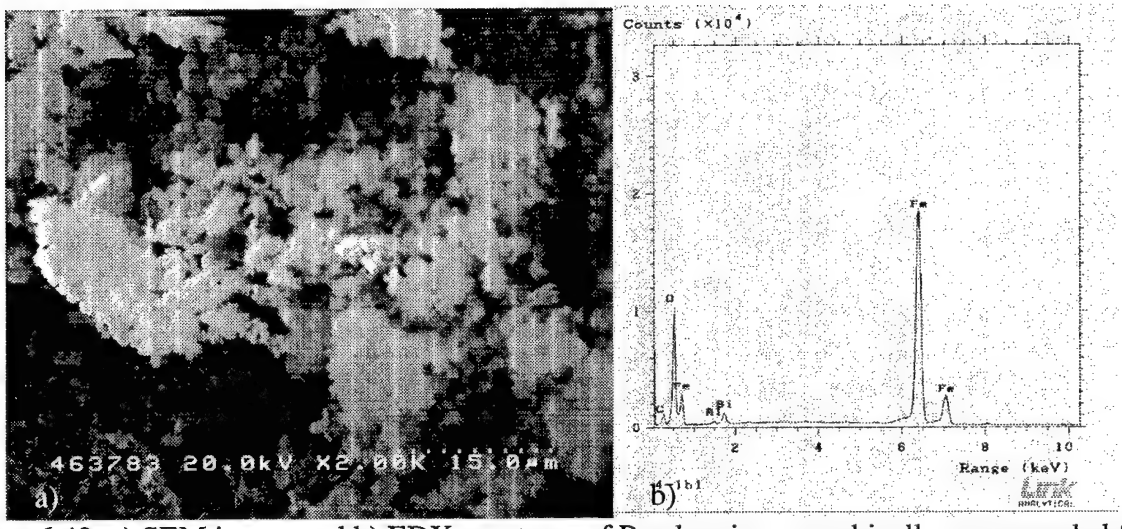


Figure 6.43. a) SEM image and b) EDX spectrum of Peerless iron, aerobically pre-corroded for 6 weeks.

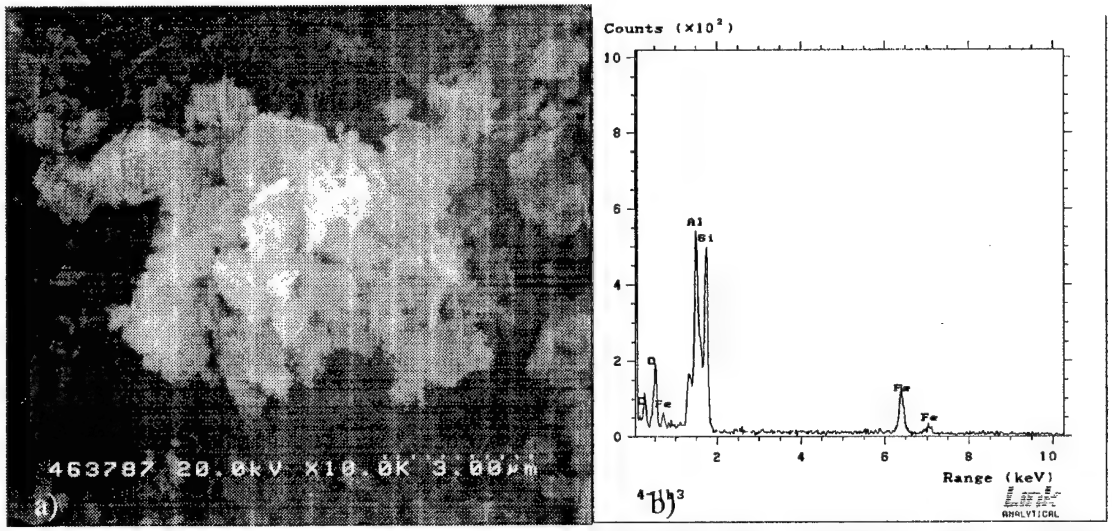


Figure 6.44. a) SEM image and b) EDX spectrum of Peerless iron, aerobically pre-corroded for 6 weeks.

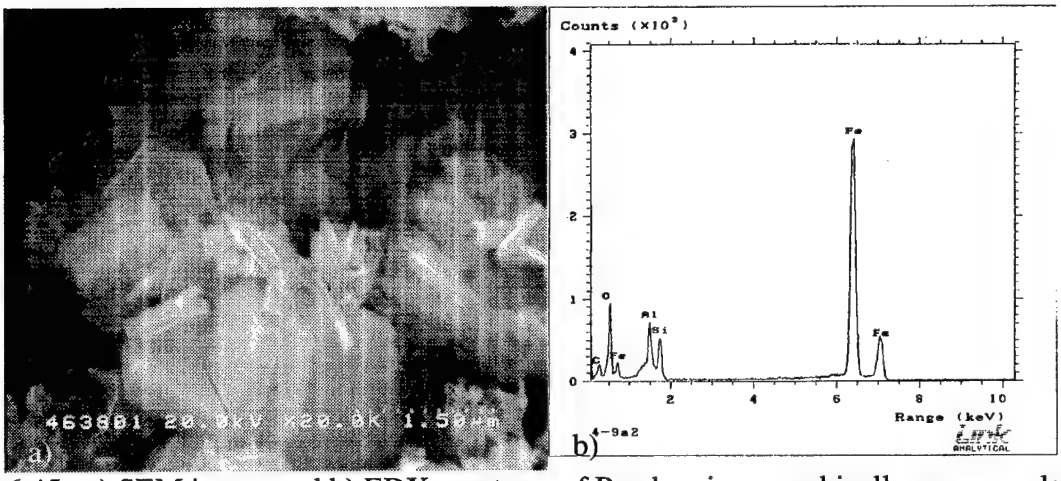


Figure 6.45. a) SEM image and b) EDX spectrum of Peerless iron aerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE water for 96 hours.

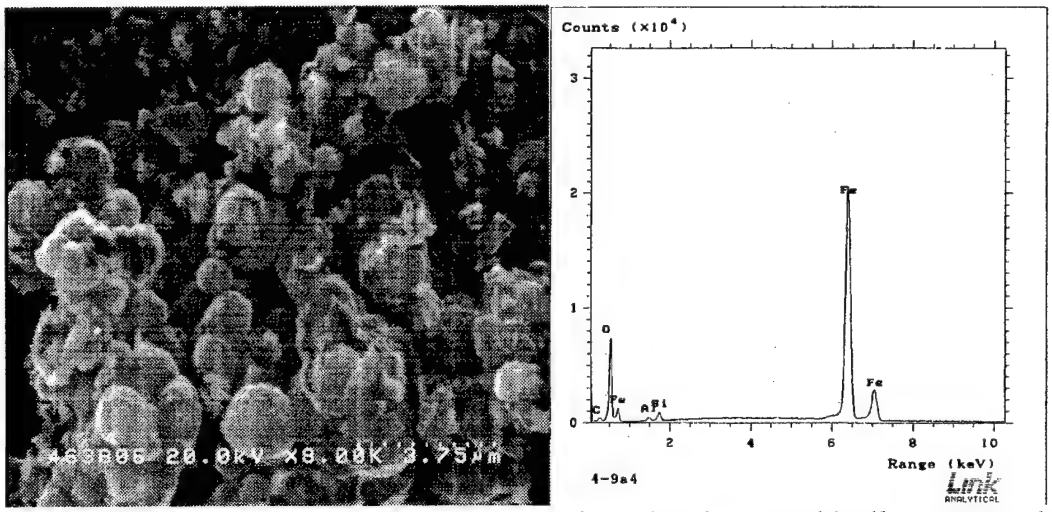


Figure 6.46. a) SEM image and b) EDX spectrum of Peerless iron aerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE water for 96 hours.

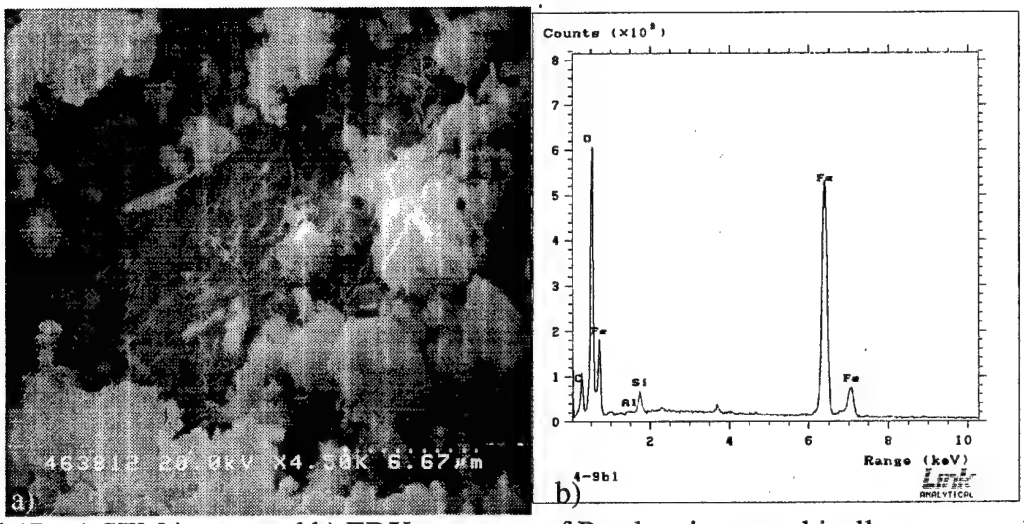


Figure 6.47. a) SEM image and b) EDX spectrum of Peerless iron aerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE water for 96 hours.

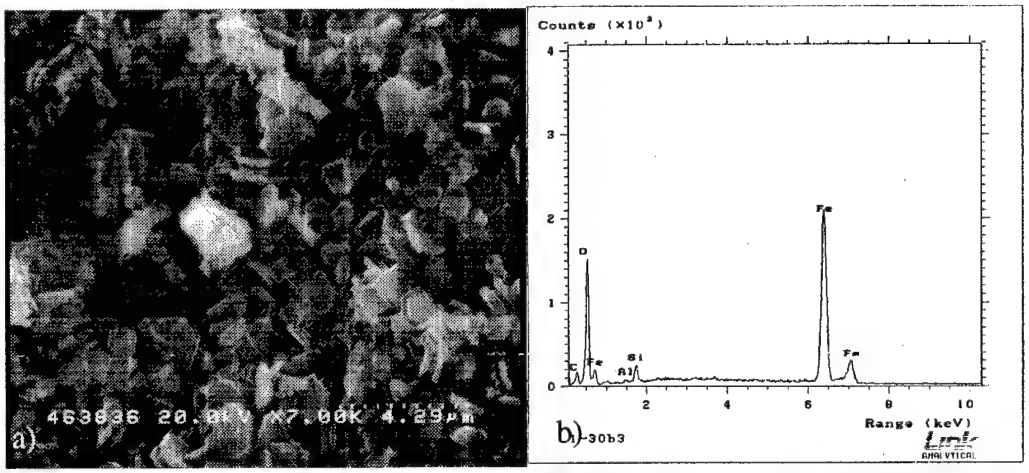


Figure 6.48. a) SEM image and b) EDX spectrum of Peerless iron, anaerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE groundwater for 336 hours.

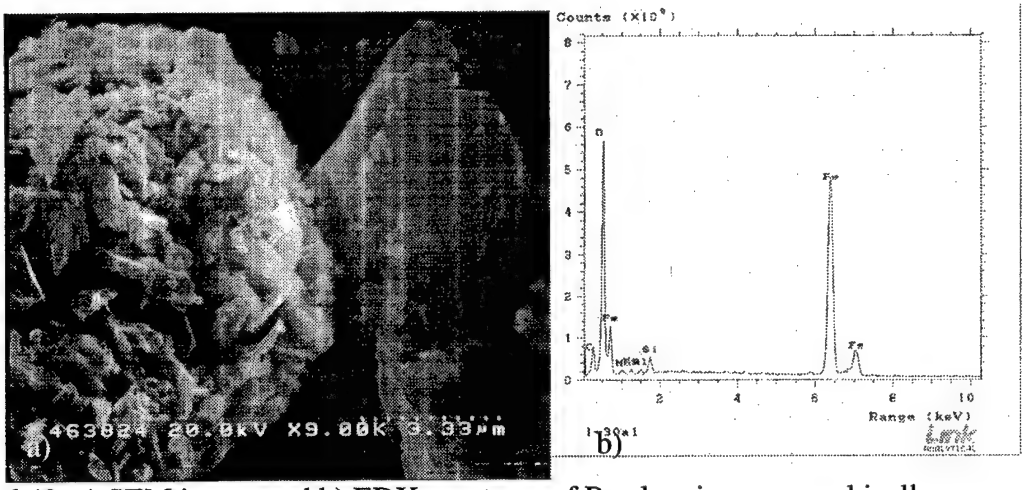


Figure 6.49. a) SEM image and b) EDX spectrum of Peerless iron, anaerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE groundwater for 336 hours.

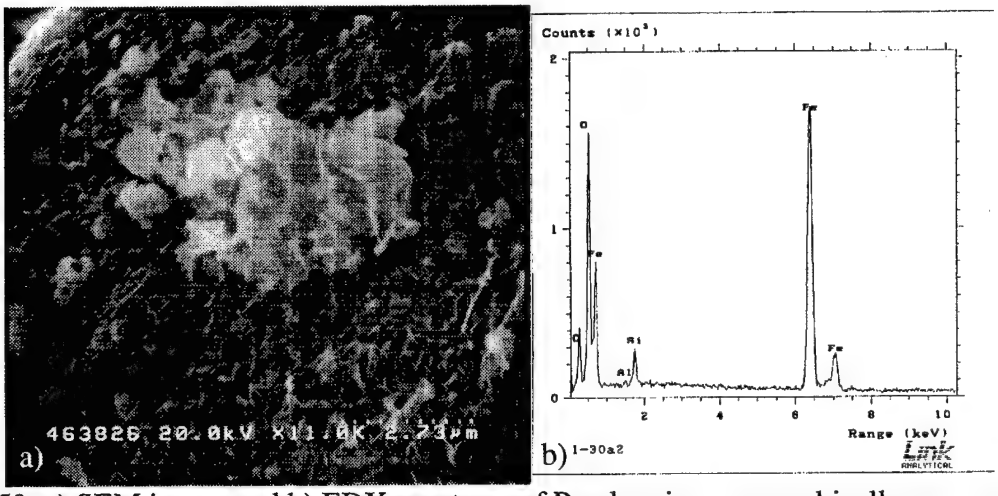


Figure 6.50. a) SEM image and b) EDX spectrum of Peerless iron, anaerobically pre-corroded for 6 weeks, then exposed to OU5 (OU12) TCE groundwater for 336 hours.

6.4.2 Rate of TCE Loss

Data generated are presented as normalized concentrations (C/Co) versus time for TCE loss for OU5 (OU12) groundwater with untreated and pre-corroded ZVI (Figure 6.51). Corrosion of the ZVI enhanced the loss of TCE (Figure 6.51). There was no significant loss of TCE from any of the controls (without ZVI). Data for the kinetic studies are in Appendix R and figures for the individual ZVI treatments are in Appendix S.

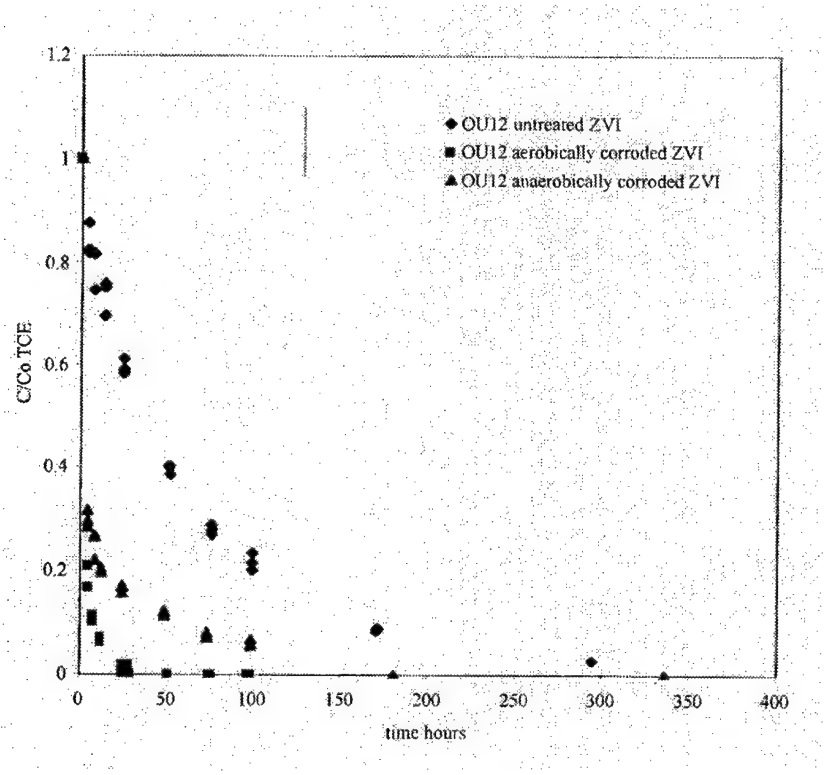


Figure 6.51. Degradation of TCE in the presence of untreated and corroded ZVI in OU5 (OU12) groundwater from Hill AFB.

The loss of TCE in the presence of ZVI is often expressed using first order kinetics (ln C/Co versus time). The relationship between ln(C/Co) versus time was curvilinear indicating that the loss of TCE on ZVI in groundwater from Hill AFB does not follow simple first order degradation over the whole time course of this study (Appendix T). The curvilinear relationship showed that the reaction rate slows with time. Other researchers have also observed a fast initial reaction followed by a slower reaction (Farrell et al., 2000 b, Agrawal et al., 2002; Arnold and Roberts, 2000; Wust et al., 1999). This change in reaction rate was attributed to saturation of reactive sites and an increase in iron surface passivation with corrosion of the ZVI.

The results for TCE loss using ZVI in the present study are described using two simultaneous first order reactions:

$$Cm = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) \qquad (9)$$

where Cm is the measured concentration of TCE in solution at time t, C_1 and C_2 are the initial concentrations of TCE associated with the fast and slow reaction, and k_1 and k_2 are the first order rate constants associated with the two reactions. The experimental data were fitted using a non-linear regression model using S-PLUS statistical software. Calculated values for the fitted variables are given in Table 6.7. Statistical parameters of fit are given in Appendix U.

Table 6.7. Proportion of TCE associated with the fast and slow degradation with ZVI and the rate constants associated with these reactions.

Treatment	C_1/Co	C_2/Co	k_1 (hr^{-1}) (t1/2 hrs)	Standard error k_1	k_2 (hr^{-1}) (t1/2 hrs)	Standard error k_2
OU12 untreated ZVI	0.12	0.88	0.556 (1.2)	0.434	0.015 (46.8)	0.0006
OU12 aerobically corroded ZVI*						
OU12 anaerobically corroded ZVI	0.74	0.26	0.644 (1.01)	0.113	0.016 (43.3)	0.0027

*fast reaction completed prior to first sampling interval of 4 hours. Data do not fit model.

Experiments using aerobically pre-corroded ZVI led to non-detectable TCE within 24 hours (Figure 6.51), so rate constants were not calculated. The fast reaction was completed prior to the first sampling interval of 4 hours (Figure 6.51). The kinetics of the reaction did not fit a zero or first order rate law, or two simultaneous first order expressions. The formation of corrosion products usually limits the rate of reaction.

Loss of TCE exposed to untreated ZVI or anaerobically pre-corroded ZVI showed similar reaction rate for the fast reaction, k_1, and the slow reaction, k_2 (Table 6.7). The reaction rates are the same but the proportion of TCE associated with the two reaction rates differ with ZVI treatment (Table 6.7). With anaerobically pre-corroded ZVI, 74% of the initial TCE concentration is associated with the fast reactions, compared with 12% for the untreated ZVI.

6.4.3 Formation of Chlorinated Degradation Products

cis-DCE was the only chlorinated degradation product detected for the reaction of TCE with ZVI under all treatment conditions of this study. No cis-DCE was detected in the fast reacting OU5 (OU12) groundwater with aerobically pre-corroded ZVI. Figure 6.52 shows the amount of DCE produced from the loss of TCE as a percentage of the initial molar concentration of TCE. DCE was detectable between 4 and 8 hours. The maximum DCE concentration was 35 to 40% (molar basis) of the initial TCE concentration. The production of cis-DCE occurred sooner and reached a higher concentration than in the untreated ZVI. Individual plots of the loss of TCE and production of cis-DCE over time are given in Appendix V.

TCE has been reported in the literature to undergo β-elimination, resulting in the production of acetylene, ethylene and ethane. The amount of cis-DCE (a product of sequential hydrogenolysis) detected in batch and column studies using high surface area, clean ZVI in deionized water is usually less than 10% (Arnold and Roberts, 2000). The large production of cis-DCE observed in this present study may be due to the use of untreated ZVI in combination with the chemistry of the groundwater at Hill AFB. Determination of this unique observation of cis-DCE production was beyond the scope of the present study. The cause is however of critical interest in designing a ZVI PRB that does not lead to the production of unwanted chlorinated by-products. Plots of the mM concentration of TCE and cis-DCE versus time for each treatment are displayed in the Appendix T.

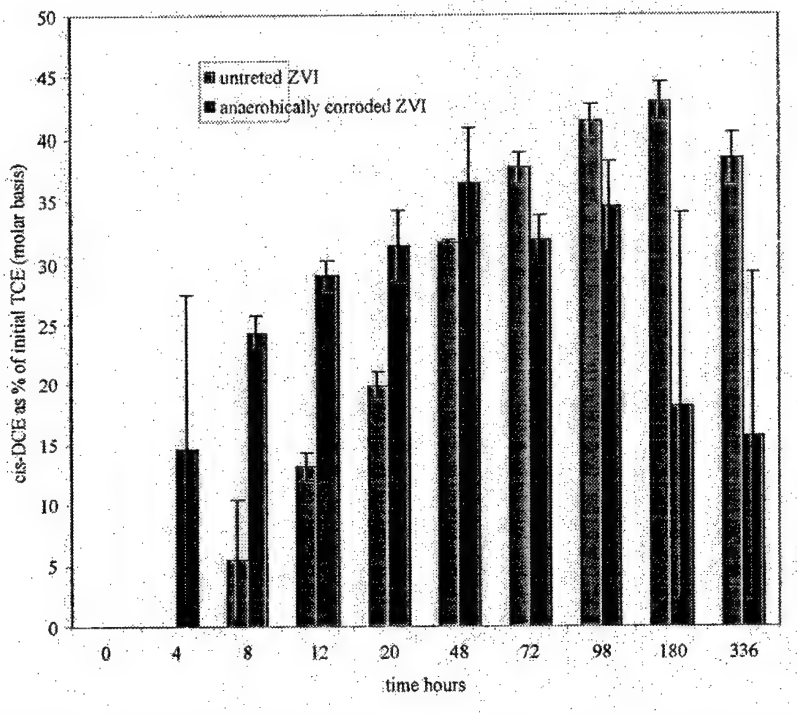


Figure 6.52. DCE production over time as a molar percentage of the initial TCE.

6.4.4 Relationship Between Corrosion Products and TCE Loss

Regardless of the pre-treatment of ZVI, all samples showed the presence of hexagonal crystals, indicative of hematite, akaganeite, or green rust. The presence of ferrihydrite was unique to the aerobically pre-corroded ZVI, as would be expected. The rate and/or extent of reaction were affected by the presence of aerobic or anaerobic corrosion products compared with untreated ZVI. The interaction with TCE and specific corrosion products is not discernable from this study.

Surface area of ZVI has a great impact in the rate of TCE loss. This experiment was designed with the same mass of ZVI in the untreated and pre-corroded reactors. Over the 6-week corrosion process, the surface area of the pre-corroded ZVI would have been significantly higher than for the untreated ZVI. Increased surface area may lead to more reactivity. Corrosion products, however, coat the ZVI, inhibiting the degradation of TCE. The increase loss of TCE in the presence of anaerobically and aerobically pre-corroded ZVI may be due to the sorption of TCE to these corrosion products and not to degradation of the TCE.

There are only a few studies in the literature that report on sorption of TCE to ZVI and corrosion products. Burris et al. (1995) demonstrated that loss of TCE and PCE from the aqueous phase in contact with ZVI was due not only to reduction processes but also to sorption of TCE to non-reactive sites on the ZVI that then sequestered this fraction of TCE from reduction reactions. Casey et al. (2000), using a miscible displacement technique, reported the linear sorption coefficient (K_d) for TCE to Fisher brand iron as 1.45 L/kg, a value similar to that reported by

Burris et al. (1995) (1.47 L/kg, as calculated by linearizing the Langmuir sorption isotherm used by Burris et al., 1995). Both authors concluded that sorption is an important process that needs to be considered in any design of a ZVI PRB. Farrell et al. (2000) studied the sorption of TCE and PCE onto simulated iron corrosion products using a sorption batch method and reported K_d vales of 0.15 L/kg and 0.18 L/kg, respectively, showing less sorption to corrosion products than to Fe^o.

If TCE is sorbed by the corrosion products, degradation by-products would not be detected in the aqueous phase. cis-DCE was produced in the anaerobically corroded ZVI in the same proportions as with the untreated ZVI (30 to 40% cis-DCE compared with the initial molar concentration of TCE). There was, however, no detection of any chlorinated breakdown product with the aerobically pre-corroded ZVI. Either all breakdown products were immediately degraded or sorbed, or little of the TCE was degraded with the only major removal mechanism of TCE being by sorption. Sorption of TCE to non-reactive sites of ZVI corrosion products means that the TCE is not degraded to non-chlorinated byproducts, but remains in the system as TCE. The conditions under which TCE would be desorbed from these sites have not been reported in the literature. Distinguishing these two removal mechanisms, degradation versus sorption, was beyond the scope of the present study. Which mechanism of removal dominates in a ZVI PRB will influence the rate and extent of treatment (is sorption treatment?) and the life span of a treatment wall.

7. CONCLUSIONS

Based on the results obtained in the various phases of this study, the following conclusions were reached.

1. From the Bachman Road Culture dilution study it was concluded that a 1:10 dilution of the mixed culture amendment provided optimal chlorinated hydrocarbon degradation in the OU5 aquifer systems. This conclusion was based on TCE degradation and intermediate product formation observed for this dilution, and was a compromise between high dilution rates, and TCE degradation rates high enough to be useful for site remediation.
2. From the MBI Granular Culture range-finding experiment it was concluded that a 1:10 dilution of this mixed culture amendment also provided sufficient TCE dechlorination capacity to be relevant to remediation system scale-up, and this dilution was used in all subsequent microcosm studies. ZVI-TCE degradation was found to be significantly greater than TCE concentration reductions in the Soil Control reactors. A soil control corrected ZVI TCE degradation rate of –0.0011/hr resulted in a TCE half-life in the ZVI amended reactors of approximately 27 days.
3. From the complete culture augmentation study it was concluded that without bioaugmentation, significant TCE degradation cannot be stimulated with the addition of carbon donor. TCE degradation rates were not significantly different than the control treatment for the Coconut Oil and Emulsified Oil amendments. Whey stimulated dechlorination but only increased the TCE degradation rate to –0.0025/d, resulting in a TCE half-life in these Whey amended reactors of 40 weeks, a time period unacceptably long for application in a site remediation scenario.
4. Water quality changes in carbon donor amended reactors without bioaugmentation lead to reducing conditions that were evident from releases of dissolved iron from the aquifer solids in the Whey and Emulsified Oil treatments, releases of dissolved arsenic from the solid phase and the

complete reduction of sulfate within 30 to 50 days in all carbon donor amended systems, and generation of large quantities of methane in the Whey amended reactors. These reduced conditions are generally thought to be sufficient to support dehalogenation reactions, but while the produced undesirable levels of iron, arsenic, and methane, they did not result in TCE degradation. It can be concluded then, that TCE dechlorination at OU5, will not be possible without bioaugmenation used along with carbon donor addition.

5. Quantitative TCE/DCE/VC data generated in the complete culture augmentation study confirm the need for and benefit of the use of combined carbon donor and microbial amendment for the stimulation of TCE dechlorination at the Hill OU5 site. Maximum TCE degradation rates were shown to increase by at least a factor 4 to 25 with the use of microbial innocula with an insoluble donor, Coconut Oil, and by as much as a factor of over 100 with the addition of microbial innocula and Whey or Emulsified Oil as the carbon source. Optimal treatment appeared to be provided by the Bachman Road culture plus Emulsified Oil based on high TCE degradation rates, high daughter product degradation rates, complete TCE/cis-DCE/VC degradation provided within 80 days of incubation, moderate iron and arsenic release rates, low methane generation rates, and increasing DOC levels produced with this treatment over time.

6. TCE reaction with ZVI in the OU5 (OU12) groundwater indicated that the reaction slows significantly over time, leading to development a dual reaction rate expression to describe TCE-ZVI interaction. The relationship has a fast degradation reaction with a half-life on the order of 1 hour, followed by a much slower degradation reaction with a half-life of more than 40 hours, possibly due to passivation of the iron surface or sorption of TCE to corrosion products associated with the ZVI solids. These reactions have the potential to limit the effectiveness of a ZVI wall at OU5, and further analysis of the long-term TCE degradation rates that can be provided by ZVI should be carefully evaluated before a full-scale iron wall is implemented at this site.

8. REFERENCES

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Appendix A. **Preliminary ZVI-TCE Degradation Study Results**

Two zero valent iron (ZVI) samples were used in this study, stock iron fillings -8 to 50 mesh, Peerless Metal Powders and Abrasive, Inc. and iron fillings 40 mesh (surface area = 0.9 m^2/g) Fisher Chemical Co. The two iron sources, Fisher and Peerless, were reacted with groundwater from OU12 under either anaerobic or aerobic conditions for 16 days. At the end of this time period there was visible corrosion products on both iron sources exposed to aerobic conditions (the samples were orange in color due to the presence of $Fe(OH)_3$). The physical characteristics of the iron sources changed under both exposure regimes (the corroded particles were described as pudding-like).

Evaluation of the various forms of iron generated in the corrosion process was carried out through a determination of the concentration of iron that was extractable using three sequential extraction procedures. Removal of exchangeable corrosion products was achieved by the addition of 20 mL of 1M ammonium chloride. Carbonate associated corrosion products were removed with 25 mL of 1M acetate buffer at pH 5. Finally 25 mL of hydroxylamine hydrochloride and hydrochloric acid solution were used to dissolve amorphous iron forms remaining in the corroded sample. Iron concentration in each filtrate was measured with flame atomic absorption, and the results of these determinations are summarized in Fig. 1A for a 7-day corrosion period.

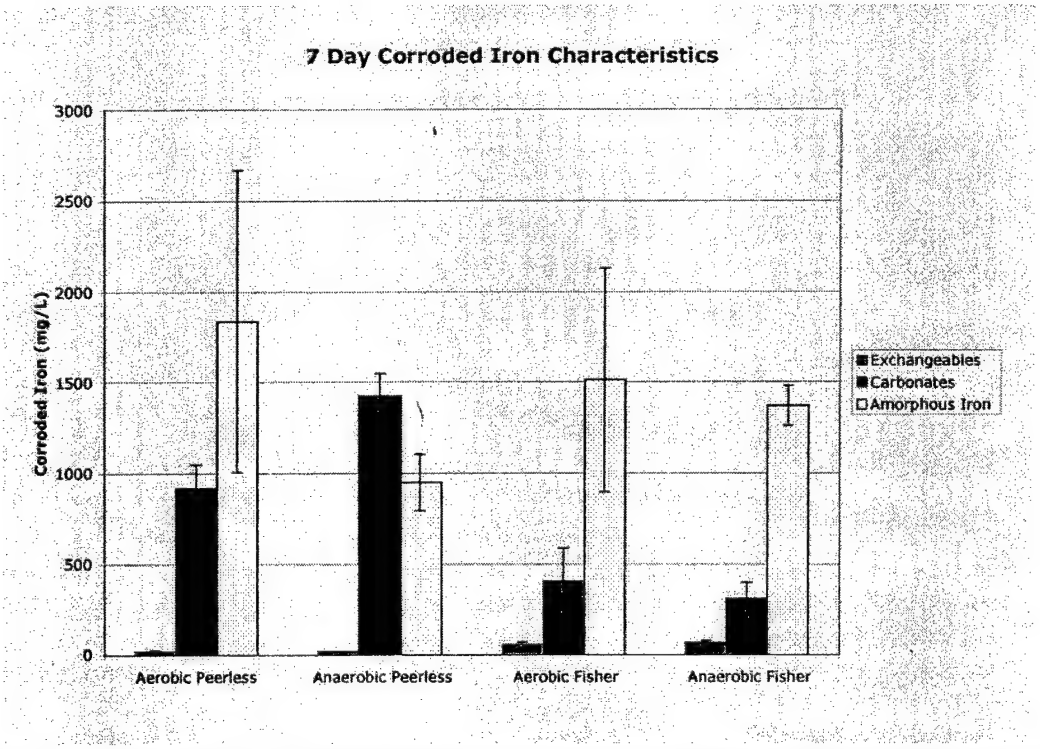

Figure 1A. Corrosion product speciation as a function of corrosion environment for a 7-day corrosion period. As indicated in Fig. 1A, pattern of iron speciation as a result of 7

days of corrosion was more dependent upon the type of iron than on the corrosion environment. The fraction of amorphous iron generated in the corrosion process was predominant for the Fisher iron regardless of corrosion environment, while it was the primary form of iron only in the aerobically corroded Peerless iron. Carbonate associated iron was the predominant species in the anaerobically corroded Peerless iron.

The distribution of iron forms during the corrosion process was also evaluated for the aerobically corroded Peerless iron over time, and the results of this evaluation is summarized in Fig 2A. Here, the distribution of iron was shown to vary significantly over the 7-day corrosion period, moving from a predominance of carbonate associated at time 0, to the predominantly amorphous forms after 7 days of aerobic corrosion.

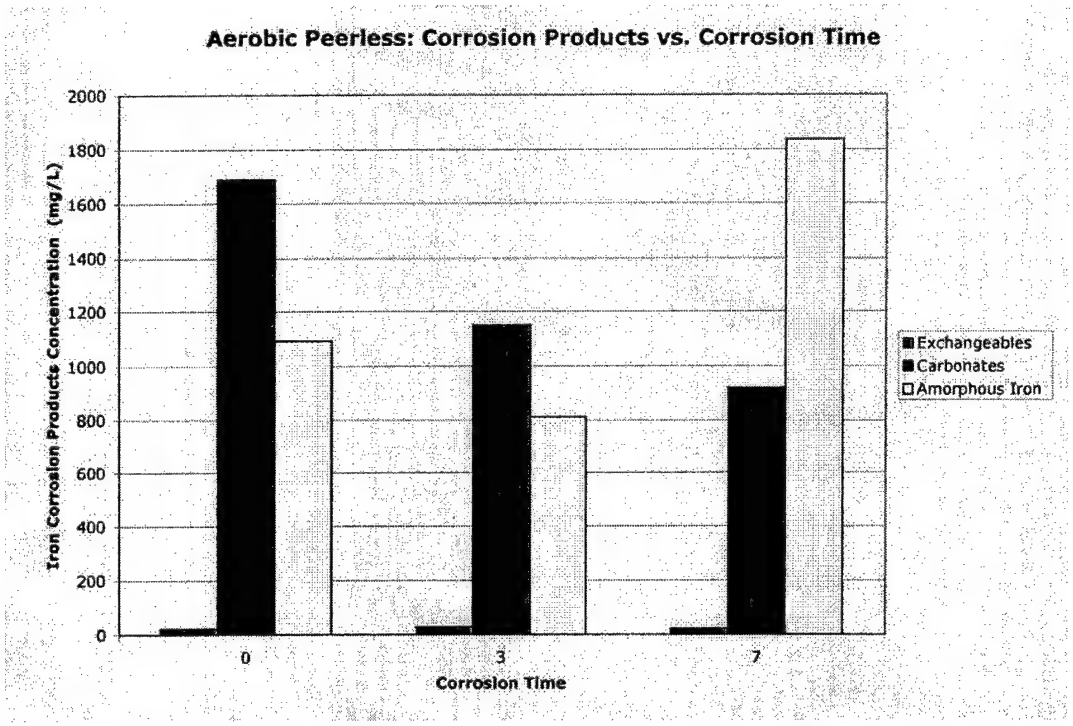

Figure 2A. Corrosion product speciation as a function of time for the Peerless iron in an aerobic corrosion environment.

After this pre-corrosion process, the supernatant was removed and 1 g of iron was added to a 25-mL crimp-top, glass headspace vials along with 9 ml of OU8 groundwater containing 5 mg/l TCE. The vials were capped with septa and incubated for varying time periods (time= 0, 4, 24, 48, 120 hours) before being sacrificed for analysis of TCE and its degradation products (DCE, VC, acetylene, ethylene, ethane) using headspace analysis. The iron sources were: untreated Fisher, aerobic corroded Fisher, anaerobic corroded Fisher, aerobic corroded Peerless, anaerobic Peerless, and no iron.

Degradation of TCE was significant for all iron sources compared with loss from the TCE only samples (13% loss). The greatest loss of TCE over the 120-hour study was with untreated Fisher iron (92% loss) and aerobically corroded Fisher iron (93% loss) (Fig. 3A). The Fisher iron, under all treatments, degraded more of the TCE than the

Peerless iron. The Fisher iron has a much larger surface area than the Peerless iron and hence shows greater reactivity. The aerobically corroded iron, Fisher and Peerless, was more reactive towards TCE than the anaerobically corroded iron. Kinetic data were fitted to first order kinetic model and calculated half-life for each treatment are presented in Table 1A.

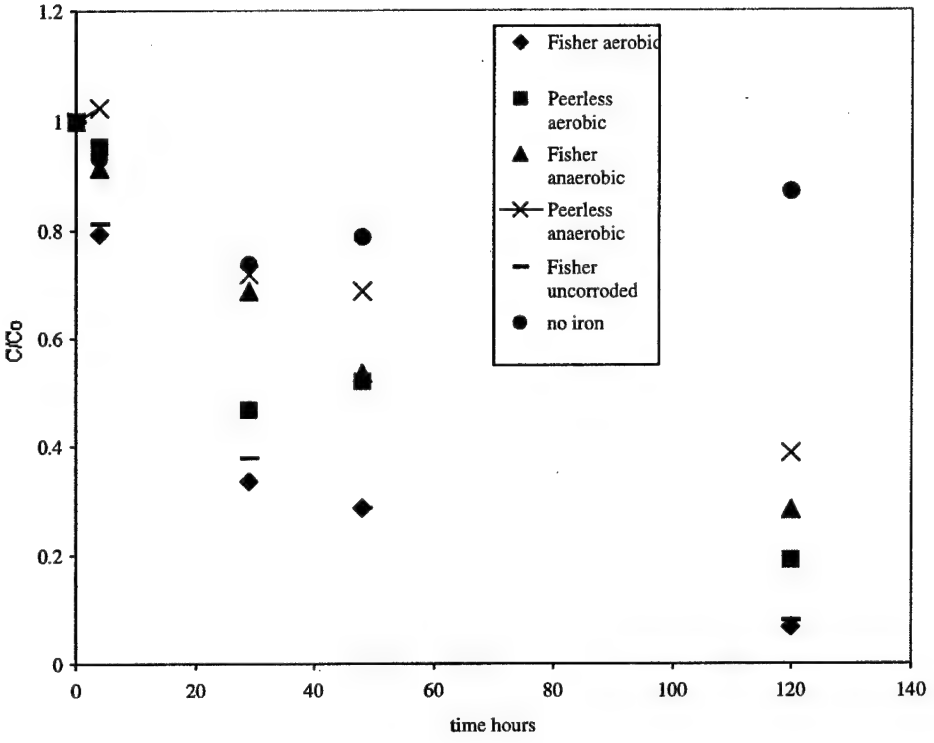

Fig 3A. Normalized TCE concentration data measured in preliminary ZVI reaction rate studies for the iron treatments listed in the legend.

Table 1A. TCE degradation half-lives based on first order rate law assumed for data presented in Figure 3A

Iron Treatment	Half-Life (hours)
Fisher	
Uncorroded	31
Aerobically Corroded	29
Anaerobically Corroded	63
Peerless	
Aerobically Corroded	48
Anaerobically Corroded	87
No Iron	Unreactive

Over 50% of the TCE that was degraded formed ethylene and ethane (Fig. 4A) regardless of the environment or the source of iron being corroded. Percent recovery of TCE plus breakdown products was 100% for untreated Fisher ZVI and aerobically corroded Fisher and Peerless ZVI. With anaerobically corroded ZVI, the recovery was 70-80%.

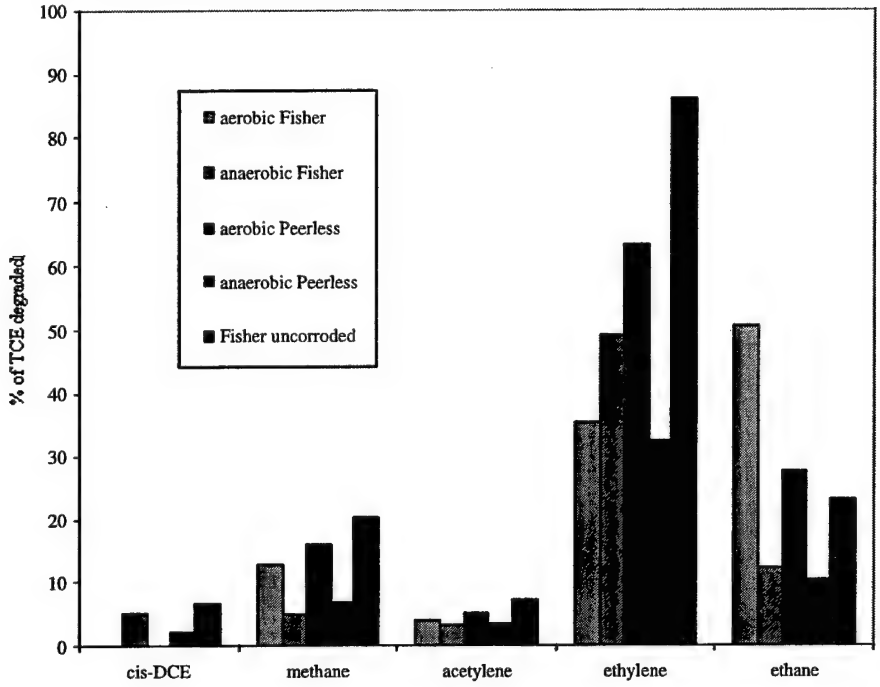

Fig 4A. Distribution of breakdown products as the percent of TCE degraded.

Potential Procedural Modification

A limitation of the procedure presently being used is that the reaction time is defined as the time of analysis. Since the reaction of TCE with ZVI is relatively fast, continued contact of TCE with the iron means continued reactions. The number of replicates and time intervals that can be examined are therefore limited by instrumentation capacity. The effectiveness of transferring aqueous samples from the reaction vessels into a analysis container without loss of TCE or transfer of reactive iron was therefore evaluated With transferring, the iron/TCE reaction would be stopped, and sample collection would then become independent of analysis time.

Two sets of microcosms were constructed as described above, one containing untreated Peerless and the other containing no iron. Following construction of these microcosms, they were sacrificed and the liquid from each was transferred to headspace vials, were sealed, and were held for various time periods up to 168 hours. The results of these analyses are shown in Fig. 5A and indicate that volatile loss was minimal during the transfer, and that there was no evidence of continued reaction due to colloidal iron/TCE reactions following sample transfer.

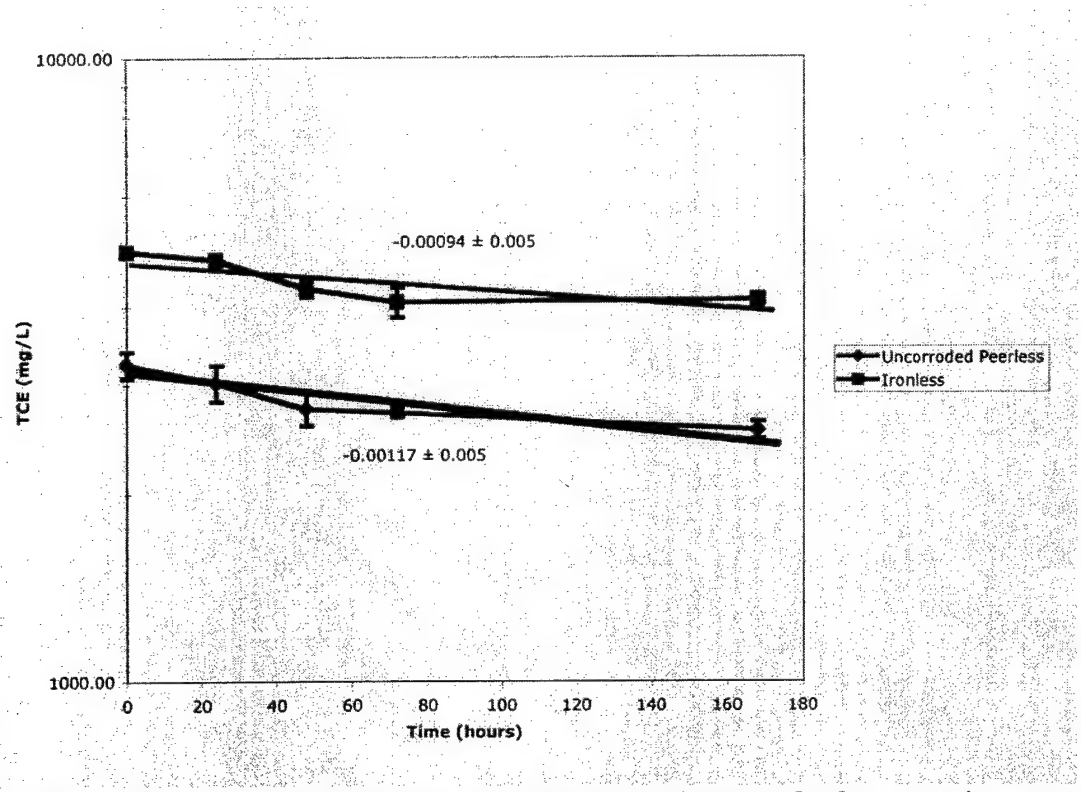

Figure 5A. TCE concentration changes over time following transfer from reaction vessel. Bars represent 95% Confidence Intervals of measurement. Equations given are slope of regression line ± 95% Confidence Interval of the slopes.

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Appendix B. SOP R-01 Standard Operating Procedures for Microcosm Construction

File Name R-01 Reactor Prep SOP V.1.2
Version No 1.2
Revision Date 9/2001

Prepared/ Revised by:

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R-1 Standard Operating Procedures for Microcosm Construction

1.0 Scope and Application

1.1 This method covers procedures for the preparation of microcosm reactors of various sizes to be used in anaerobic biodegradation experiments.

1.2 This method is applicable to batch microcosm reactors of 20-mL and 120-mL total volume for simulated groundwater systems in which various terminal electron accepting processes are being evaluated.

2.0 Summary of Method

2.1 Microcosms of 20-mL and 120-mL total volume are constructed to contain aquifer material and groundwater from a specific contaminated site of interest. These microcosms are prepared with a fixed soil to water volume ratio, producing a fixed volume of headspace in them prior to being sealed.

2.2 Additional nutrient, carbon donor, and electron acceptor solutions are added throughout the construction process depending upon the specific treatment being constructed.

2.3 TCE-amended groundwater is added to the reactors, and the reactors are sealed and incubated for specified time intervals before they are sacrificed for analyte determinations using appropriate inorganic or organic analysis SOPs specified by the UWRL.

3.0 Apparatus and Materials

3.1 Headspace vials, 20-mL and 120-mL, with PTFE/Silicone caps.

3.2 Contaminated soil and groundwater (from Hill AFB, OU5).

3.3 103 ± 2 °C laboratory drying oven.

3.4 Yeast extract, powder form (spiking solutions to yield final microcosm concentrations of 20 mg yeast/L of groundwater, see Table 1).

3.5 Carbon donor, soluble and insoluble (See SOP R-02 for donor preparation).

3.6 Spatula (dual ended, with flat edges).

3.7 Repipettes (100 µL, 1,000 µL, 5 mL variable volume; 200 µL fixed volume).

3.8 Dispensing bottle repipettes (10 mL, 100 mL).
3.9 Modified spatula and measuring spoon (Kitchen Art 5 mL and 15 mL).
3.10 TCE spiking solution (variable concentration, see Table 1).
3.11 Mixing bowls and mixing utensils.
3.12 Top loading balances.
3.13 Aluminum foil.
3.14 0.5 g measuring spoons.

4.0 Procedure

4.1 General Procedures for All Reactors.

4.1.1 Prepare a high concentration TCE spiking solution by diluting neat TCE in methanol. Suggested concentrations are 4,000 mg/L. The addition of 2.5 mL of this solution to 1 L of groundwater yields the desired final TCE groundwater concentration of 10 mg/L.

4.1.2 Prepare the soluble and slow-release carbon donor mixtures according to SOP R-02.

4.1.3 Prewash and autoclave all microcosm bottles and caps to ensure that they are scrupulously clean prior to use.

4.1.4 Collect at least three samples of aquifer soil material to be placed within the microcosms, and determine a dry soil weight for each sample using the following procedures:

4.1.4.1 Pre-weigh aluminum weighing pans on an analytical balance and record their weight to the nearest 0.0001 g. This is the *pan weight*.

4.1.4.2 Place a representative sample of the aquifer material into the weighing pans and record their weight to the nearest 0.0001 g. This is the *pan +wet soil weight*.

4.1.4.3 Place the samples in a 103 °C drying oven and dry samples to a constant weight. Record this weight to the nearest 0.0001 g. This is the *pan + dry soil weight*.

4.1.4.4 Determine the weight of water in each sample using the following calculation: wt. water, g = (*pan +wet soil weight*) - (*pan + dry soil weight*).

4.1.4.5 Determine the dry weight of soil in each sample using the following calculation: dry soil wt., g = (*pan + dry soil weight*) - (*pan weight*).

4.1.4.6 Determine the percent water content of the samples using the following calculation: % water = (wt. water)/(dry soil wt.).

6.2.1.1 Determine the soil wet weight factor for use in determining the dose of soil to each reactor using the following calculations:
Soil wet wt. = Dry soil wt. (1 + % water);
Soil wet weight factor = (1 + % water).

4.1.5 Determine the wet weight of soil to be dispensed into each of the reactors based on the measured soil % water and a desired weight of dry soil in each reactor. This involves the determination of the appropriate setting on modified spatula to dispense the desired weight of wet soil for each reactor using the following procedures:

4.1.5.1 Scoop moist soil into modified spatula to desired position that approximates the desired wet weight of soil.

4.1.5.2 Clean debris off the sides and bottom of the spatula.

4.1.5.3 Place a weighing paper on a top loading electronic balance and tare.

4.1.5.4 Weigh the amount of soil placed in the weighing pan and make adjustments as necessary to reach the desired wet weight.

4.1.5.5 Once the spatula is adjusted to dispense the proper weight, weigh 10 samples dispensed with this spatula and record the weights. If the 95% CI of these weights is within 5% of the desired weight, continue with the reactor construction process. If the 95% CI is greater than 5%, repeat this step until a consistent wet of wet soil can be placed within each reactor.

4.1.5.6 Alternatively, weigh each soil aliquot placed into the microcosms, assuring that each is within ± 10% of the desired value.

4.2 Procedures for 20-mL Reactors.

4.2.1 For slow-release carbon donor treatments, dispense 0.6 g ± 0.1 g of pre-loaded sand carrier material into each reactor. This carrier should contain approximately 1 mg slow-release donor/g carrier as per SOP R-02.

4.2.2 For soluble carbon donor treatments, dispense 0.2 mL of variable concentration donor solutions to each reactor using a fixed volume 200 µL repipette.

4.2.3 Dispense the appropriate wet weight of soil into each reactor based on a desired dry weight in each of 3 g.

4.2.4 Add 0.2 mL of the appropriate nutrient solution (Table 1) into each reactor using a fixed volume 200 µL repipette.

4.2.5 For the abiotic treatments, cover the microcosms with pre-cut aluminum foil and place in an autoclave for ≥ 15 min at 121° C.

4.2.6 Dispense 9.0 mL of site TCE-amended groundwater (8.8 mL for nutrient amended treatments) into each reactor using a bottle dispensing repipette, and mix the rectors by inverting them six times to completely mix their contents.

4.2.7 Place the mixed reactors in a constant temperature room at 15 °C in sealed, inerted bags. Incubate reactors in the dark for the desired time before sacrificing them for analysis using appropriate inorganic or organic analysis SOPs specified by the UWRL.

4.3 Procedures for 120-mL Reactors.

4.3.1 For slow-release carbon donor treatments, dispense 5.0 g ± 0.1 g of pre-loaded sand carrier material into each reactor. This carrier should contain approximately 1 mg slow-release donor/g carrier as per SOP R-02.

4.3.2 For soluble carbon donor treatments, dispense 0.2 mL of variable concentration donor solutions to each reactor using a fixed volume 200 µL repipette.

4.3.3 Dispense the appropriate wet weight of soil into each reactor based on a desired dry weight in each of 25 g.

4.3.4 Add 1.0 mL of the appropriate nutrient solution (Table 1) into each reactor using a variable volume 5 mL repipette.

4.3.5 For the abiotic treatments, cover the microcosms loosely with their caps and place in an autoclave for ≥ 15 min at 121° C.

4.3.6 Dispense 75.0 mL of site TCE-amended groundwater (74.0 mL for nutrient amended treatments) into each reactor using a bottle dispensing repipette, and mix the reactors by inverting them six times to completely mix their contents.

4.3.7 Place the mixed reactors in a constant temperature room at 15 °C in sealed, inerted bags. Incubate reactors in the dark for the desired time before sacrificing them for analyte determinations using appropriate inorganic or organic analysis SOPs specified by the UWRL.

5. Quality Control

5.1 The weights of soil and slow release donor carrier should be controlled to within ± 5% of the desired values shown in Table 1 if a calibrated dispensing device and QC verification samples are being used during reactor construction. If each aliquot of soil or carrier material added to the microcosms is measured during reactor preparation, a larger ± 10% interval can be accepted. All weights should be recorded and verified that they meet acceptance criteria.

5.2 TCE check samples will be collected during the preparation of the microcosm reactors. Check samples will consist of 9 mL aliquots of TCE-amended groundwater placed in headspace vials using the bottle repipetters, and will then be immediately sealed and analyzed for TCE concentration. These samples will be generated for each type of microcosm produced during construction (20-mL and 125-mL reactors) before groundwater is dispensed into the microcosms, at the midpoint of the dispensing process, and at the end of microcosm preparation so that TCE concentrations within the reactors can be verified during the reactor construction process.

5.3 Visual inspection of volume dispensing devices should be carried out on an on-going basis prior to their use to ensure the proper volume of each component of the reactors is put into them during construction.

5.4 Reactor preparation QA/QC log sheets should be produced during the process of reactor construction to ensure that the appropriate weights/volumes of soil/donor are placed within the correct reactors (see Table 2). If errors or omissions are noted, suspect reactors should be replaced by new units.

6.0 Corrective Action

6.1 If using a calibrated dispensing device and QC verification samples:

6.1.1 If the QC weight is within the ± 5% control limit, record the weight on the reactor preparation QA/QC sheet and continue with the next batch of microcosms.

6.1.2 If the QC weight is outside the ± 5% control limit, record the weight on the reactor preparation QA/QC worksheet, and reconstruct all of the reactors that were made since the last QC weight sample was within ± 5% of the desired value.

6.2 If weighing each soil or slow release carbon carrier sample before putting them into the microcosms:

6.2.1 If the QC weight is within the ± 10% control limit, record the weight on the reactor preparation QA/QC sheet and continue with constructing the next microcosm.

6.2.2 If the QC weight is outside the ± 10% control limit, adjust the weight by adding or removing material until its weight falls within the ± 10% control limit. Record the weight on the reactor preparation QA/QC worksheet, and continue building the next microcosm reactor.

6.3 If the volume dispensing apparatus are not adjusted to the proper volumes shown in Table 1, these apparatus should be adjusted to the proper settings, and any reactors constructed with the incorrect volume of reagents should be reconstructed before new microcosm construction begins.

6.4 TCE groundwater concentration check samples will be used to verify TCE concentrations within reactors during their construction.

6.4.1 If the initial TCE check sample is greater than 15% from the desired concentration, microcosm construction should not begin, and the solution should be remade and analyzed to ensure the proper concentration within the reactors.

6.4.2 If the intermediate or final check sample is greater than 10% from the initial check sample, another check sample should be produced and run to verify TCE concentrations being dispensed from the repipetter. If the second sample is not within 10% of the initial check sample, the TCE-amended groundwater should be remade and tested to verify its concentration before further microcosm construction continues. All reactors since the last acceptable check sample should be reconstructed using the newly prepared TCE-amended groundwater before new microcosms are created.

7.0 Record Keeping and Storage

The reactor construction QA/QC logs will be kept for future reference and data interpretation efforts. These data will be kept on file for a minimum of 5 years from the date the report is sent to the client.

Table 1. Summary of Reactor Components in Microcosm Experiments.

Reactors	Soil	Ground Water	Soluble Carbon	Yeast Solution	Insoluble Carbon
20 mL Carbon Donor Dechlorination Reactors (biotic or abiotic)	3.0 g Soil Dry Weight	8.8 mL TCE Amended (10 mg/L) Groundwater	200 µL	200 µL of 900 mg/L solution	0.6 g
20 mL Carbon Donor Dechlorination Reactors (biotic or abiotic) without Nutrient Admendment	3.0 g Soil Dry Weight	9.0 mL TCE Amended (10 mg/L) Groundwater	200 µL		0.6 g
125 mL Metals Release and Water Quality Indicator Reactors (biotic or abiotic)	25 g Soil Dry Weight	74.0 mL TCE Amended (10 mg/L) Groundwater	200 µL	1 mL of 1500 mg/L solution	5.0 g
125 mL Metals Release and Water Quality Indicator Reactors (biotic or abiotic) without Nutrient Admendment	25 g Soil Dry Weight	75.0 mL TCE Amended (10 mg/L) Groundwater	200 µL		5.0 g

Table 2. Example Reactor Construction QA/QC Sheet.

TREATMENT 3 - Low MP Oil				
Soil Loading	3.7 to 3.8 g			
Donor Loading	0.59 to 0.61 g	1 mg C/g		
Date		Bucket #		
Builder				
				Reactor Label
Bottle #	Donor Wt (g)	Soil Wt (g)	Nutrient (¦)	(Treat ¦ Nut ¦ Bio ¦ Event ¦ Rep)
1				
2				
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4				
5				
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Appendix C. SOP O-1 Halogenated VOCs by GC/MS Using Modified EPA Methods 5021 and 8260B

File Name VOC/GCMS SOP Ver.1.1
Version No 1.1
Revision Date 10/2001

Prepared/ Revised by:

_____

Coreen R. Crouch, Research Technician III

Approval:

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William J. Doucette, Ph.D.
Organics Manager

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Joan McLean, QA Officer

O-1 Standard Operating Procedures for Halogenated VOC Analysis by GC/MS Using Modified EPA Methods 5021 and 8260B

1.0 Scope and Application

1.1 This method describes the procedures for the determination of the concentration of volatile halogenated organics in the aqueous phase of microcosms by headspace capillary column/gas chromatography/mass spectrometer (GC/MS).

1.2 The SOP is based on procedures documented in Methods 5021 and 8260B, U.S. EPA (1996).

1.3 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, and leachates.

2.0 Method Summary

2.1 Microcosm aqueous phase concentrations of halogenated volatile hydrocarbons with high Henry's Law constants are determined indirectly by measuring their equilibrium headspace gas concentrations after heating crimp-seal headspace vials to a specific temperature using an automated headspace analyzer interfaced to a GC/MS.

2.2 A sample of the vapor phase is removed from the vial headspace through a sample transfer line and injected into a capillary gas chromatography column connected to a MS. The column is temperature programmed to separate the analytes.

2.3 Separated analytes are then identified and quantified by an electronic data acquisition system collecting data from the MS.

3.0 Interferences

3.1 Major contaminant sources are volatile materials in the laboratory and impurities in the reagent water and inert purge gas. Analyses of laboratory reagent blanks provide information about the presence of contaminants.

3.2 Interfering contamination may also occur when a sample containing low concentrations of analytes is analyzed immediately after a sample containing relatively high concentrations of the analytes. It is recommended that one or more laboratory reagent blanks be analyzed after the analysis of a highly contaminated sample to check for cross contamination.

3.3 The sample matrix itself can cause severe interferences by one of several processes or a combination of them. This includes interfering organic and inorganic species dissolved in the aqueous phase that impact the equilibrium that should be established within the headspace analyzer.

3.4 Interference can also be caused by soils high in organic carbon which can inhibit the partitioning of the volatile target analytes into the headspace making their recovery from the matrix low.

3.5 To overcome this "matrix effect," surrogates can be added to a matrix and analyzed to determine the percent recovery under a given matrix effect. All standards and calibration control samples should also be made up using a water phase as close as possible to the actual water phase for which the analysis is being conducted to minimize the impact of this matrix effect on subsequent results.

4.0 Safety

The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Pure standard and stock standard solutions of these analytes should be handled in a hood.

5.0 Apparatus and Equipment

5.1 GC/MS - HP6890 GC equipped with an HP5973 MS detector, a Tekmar 7000HT Headspace Analyzer with autosampler carousel, and HP's EnviroQuant Chemstation G1701AA version A.03.00 data acquisition and analysis system installed on a Pentium PC.

5.1.1 GC Column: J&W DB-624, 20 M x 0.179 mm, 1µm film thickness.

5.1.2 Carrier gas: Helium at 0.8 mL/min (8 psi).

5.1.3 Oven temperature program (total run time = 15.1 min.):
- Initial temperature 35 °C, hold 4 min.
- Ramp at 15 °C/min to 200 °C, hold 0.10 min.

5.1.4 Split/splitless inlet vent flow: 24.3 ml/min

5.1.5 Split ratio: 35:1

5.1.6 Tekmar autosampler setting parameters
- Sample loop - 1 mL loop
- Platen/Sample temperature - 50 °C
- Platen equilibrium time - 0 min
- Vial size - 22 mL
- Mixer - On 5 min, power of 8, stabilize for 1 min
- Pressurize Setting - 22 psi
- Pressurize Time - 0.20 min
- Pressure Equilibrium Time - 0.08 min
- Loop fill time - 0.4 min
- Loop equilibrium time - 0.08 min
- Inject - 1.0 min
- Injections per vial - 1
- Sample loop temperature - 180 °C
- Line temperature - 180 °C
- GC cycle time - 30 min

5.2 Tekmar autosampler procedures

5.2.1 Samples, standards and QC samples are placed into various positions in the autosampler, and these positions and sample identifications are logged into the sample analysis logbook and computer system.

5.2.2 Reserve the first few slots for standard and QC samples. Prepare the standards, ICV, CCV and LCS. Use sparged site groundwater as the liquid medium; do not use reagent water.

6.0 Reagents

6.1 Reagent water: Deionized water that is free of analytes. Water should be filtered through a 0.2 µm filter. At least one method blank should be performed for each batch of samples.

6.2 Methanol: Optima grade.

6.3 Stock standard solutions: QTM Volatile Halocarbons Mix, 2,000 mg/L in methanol, purchased from Supelco, Cat. No. 48001, or other qualified vendors

6.4 Matrix spikes: A continuing calibration verification (CCV) matrix spike at mid-level of the initial calibration is used for VOC analysis. To make the control checks, a standard from a separate source is used to make stock solutions. The ICV, CCV, and LCS are all made from a source other than the initial calibration standard. The maximum lifetime for non-gas standards in 1 mL vials is 6 month or sooner if comparison with initial calibration curve or check standards indicates a problem.

6.5 Working standard solutions: From the stock standard solutions, make the following working standard solutions at 50 and 500 mg/L in methanol:

6.5.1 To make a 50 mg/L solution, use a pipette and accurately transfer 2.0 mL of methanol into a 4 mL screw cap vial. With a gas-tight syringe, add 50 µL of the stock standard solution and cap tightly.

6.5.2 To make the 500 mg/L solution, add 500 µL of the stock standard solution to 2.0 mL of methanol.

6.5.3 These working standards should be kept in the freezer and are used to prepare standards for generating calibration curves.

7.0 Sample Collection, Preservation and Storage

7.1 Samples for headspace analysis are taken from the 20 mL microcosm reactors that were prepared according to standard operating procedure SOP R-1. These microcosms consist of 20 mL glass headspace vials with crimp sealed lids and gas tight septa. These microcosms are removed from the constant temperature incubation room at appropriate sampling intervals, and are then immediately ready for placement on the autosampler platen for headspace analysis.

7.2 If sample analysis cannot be carried out within 24 hours of the reactors being removed from the constant temperature room, they should be held at 4 °C ± 2 °C prior to analysis.

7.3 If sample analysis cannot be carried out within 48 hours, the microcosms should remain in the constant temperature room until headspace analysis of them can be carried out within a 24 hour period.

8. Procedure

8.1 Operating Conditions

8.1.1 Make sure that all gas cylinders have a tank pressure of 300 psi or greater prior to starting the analysis. Change gas cylinders at or before the 300 psi gauge level.

8.1.2 Confirm that the carrier gas pressure through the column is set at 8 psig.

8.1.3 Confirm that the ion source vacuum is between 4 and 6 x 10^{-5} torr at an oven temperature of 200 °C.

8.1.4 Confirm that the tune is complete and verified.

8.1.5 Confirm that the column initial temperature is 35 °C.

8.2 Initial Calibration

8.2.1 To ensure the utmost MS performance, the GC/MS system must be hardware tuned. A full target tune is run and saved every 7 days. Targets are as follows: mass 69 = 100%, mass 50 = 1, mass 131 = 55, mass 219 = 45, mass 414 = 3.5, and mass 502 = 2.5. A quick tune is run and saved every 12 hours the instrument is in use, or otherwise every day.

8.2.2 Initial calibration standards should be prepared over a range of concentrations expected in the sample or should define the working range of the detector. One of the standards should be at a concentration near, but above, the method detection limit. The highest or the lowest calibration point can be dropped for some compounds if the calibration linearity cannot meet method requirement.

8.2.3 A minimum of seven standards are prepared just before system calibration by using the calibration working standard and site groundwater purged with nitrogen. Volumetric flasks should be used for water volumes larger than 25 mL. For water volume less than 25 mL, 5-mL to 30-mL syringes or pipettes should be used for accurate volume dispensing.

8.2.4 Since the calibration standards are prepared in a water solution, they should be loaded onto the autosampler and analyzed immediately after preparation.

8.2.5 Standards and QC samples are placed into various positions in the auto injector, and these positions and sample identifications are logged into the sample analysis logbook and computer system.

8.2.6 Edit a sequence in the EnviroQuant Chemstation software which corresponds to the sequence in the Tekmar 7000HT and start the analysis procedures.

8.2.7 Using the determined chemical response and concentration of each calibration standard, a calibration curve can be prepared with a linear curve fit. The EnviroQuant software will do this automatically based on specified programming procedures.

8.2.8 Linearity. The correlation coefficient of the multipoint external calibration curve is used to evaluate the linearity and validity of the calibration curve for each analyte. The correlation coefficient of the calibration curve regression (r) must be greater than or equal to 0.99 for the calibration curve to pass QC.

8.3 Sample Analyses

8.3.1 Allow the treatment microcosms to warm to ambient temperature before analysis.

8.3.2 Samples, standards, and QC samples (blanks, ICVs, LCSs, and CCVs as indicated in Table 1) are placed into various positions in the headspace autosampler. The sample order should allow for the initial analysis of a method blank and system calibration check sample prior to sample analysis.

8.3.3 Edit a sequence in the EnviroQuant program. Make sure the sequence in the auto injector matches that in EnviroQuant. Log the vial positions and their sample identifications in the sample analysis logbook and computer system. Press "start" on the auto sampler to start the sequence.

8.3.4 By inspection of the method blank and ICV, verify that the system is free of background contamination and is in calibration. If required, recalibrate as described in Section 8.2.

— The width of the retention time window is used to make identifications of actual retention time variations of standards

over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

8.4 Data Analysis. Calculation, and Reporting

8.4.1 An analyte is identified by identification of the target and qualitative ions, along with the retention time assigned to the compound. Table 2 summarizes the analyte target and qualitative ions and their relative response and percent relative uncertainty based on the analyte list of importance in the Hill AFB VegOil study.

Table 1. QC samples used during analysis.

QC Sample Label	QC Sample Name	Sample Description
Blank	Instrument Blank	Groundwater from OU-5, HAFB purged with N_2
ICV	Initial Calibration Verification	Midpoint calibration measurement in purged OU-5 groundwater
LCS	Laboratory Control Sample	Midpoint calibration measurement in deionized water
CCV	Continued Calibration Verification	Midpoint calibration measurement in purged OU-5 groundwater

Table 2. Analyte target and qualtiative ions, their relative response and percent relative uncertainty.

Analyte	Target Qualitative Ion(s)	Relative Response Target Qualitative Ion(s)	% Relative Uncertainty Target Qualitative Ion(s)
vinyl chloride	62	100	20
cis-dichloroethene	61/96	100/75	20/20
trans- dichloroethene	61/96	100/60	20/20
trichloroethene	130/95/60	100/95/50	20/20/20

8.4.2 An analyte could also be identified by comparison of the sample mass spectrum with the mass spectrum of a standard of the suspected compound (standard reference spectrum). These standard reference spectra are obtained through analysis of the calibration standards and are confirmed by the EnviroQuant program software library.

8.4.3 Elution of sample components (RT) must be within ± 5% of the RT of the standard component to verify identification of unknows within the sample chromatograms. Table 3 lists the approximate retention times of each compound of interest in this Hill AFB VegOil project.

Table 3. Retention times for VOCs from analysis of known standard solutions using the current SOP method.

Compound	Approximate Retention time (min.)
vinyl chloride	1.47
cis-dichloroethene	3.56
trans-dichloroethene	5.03
trichloroethene	6.85

8.4.4 To obtain the correct peak areas, proper integration parameters should be used for standard and sample integration. The EnviroQuant program auto integrate function should be used for the integration of all peaks. In principle, a special integration file can be assigned to both the target and qualifier ions for each compound.

8.4.5 Report results in mg/L for each analyte.

9. Quality Control

9.1 QC Samples

Method blank (MB), initial calibration verification (ICV), continuing calibration verification (CCV), and laboratory control samples (LCS)

should be analyzed on a daily basis or for each analytical batch (20 samples or less), whichever is more frequent.

9.2 QC Limits

9.2.1 Before any samples are analyzed, it should be demonstrated with a method blank, that the system is reasonably free of contamination that would interfere with the determination of any analytes of interest.

9.2.2 The initial calibration curve should have a correlation coefficient, r, of at least 0.99 with a minimum of three concentration points plus a blank. The control limit for initial calibration verification (ICV) is 65% - 135% based on control chart records for this analysis.

9.2.3 Perform the daily or continuing calibration verification (CCV) by measuring the midpoint calibration concentration before analysis of every 10 samples. The control limit for the CCV is 70%-130% based on control chart records for this analysis.

9.2.4 The analyte retention times in the CCV sample must also be evaluated immediately after or during data acquisition. If the retention time for any analyte changes by more than three standard deviations of the retention time observed in generating the calibration curve, the chromatographic system must be inspected for malfunctions and corrections must be made, as required.

9.2.5 The recoveries of the analytes in LCSs should be within the control limit of 75%-125%.

9.2.6 All calibration and method blanks should have concentrations of analytes less than the practical quantitation limit (PQL).

9.3 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL(µg/L)	Date	PQL (µg/L)	Source
vinyl chloride	1.14	9/7/01	1.9	UWRL
cis-dichloroethene	0.61	9/7/01	1.0	UWRL
trans-dichloroethene	0.91	9/7/01	1.5	UWRL
trichloroethene	0.65	9/7/01	1.1	UWRL

10.0 Corrective Action

10.1 If the method blank (or instrument blank) indicates a result higher than the PQL, the containers, reagents, and analytical system should be carefully examined and cleaned until the background disappears before samples can be analyzed.

10.2 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

10.3 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

10.4 If there is not enough sample for a MSD or even for a MS, the LCS/LCSD may be used for the QC report.

10.5 If one of the recoveries of the MS and MSD is outside of the control limit check the average and RPD of the MS and MSD. If both the average and the RPD value are within the control limit, the results are acceptable. If the average of the MS and MSD is out of control but the RPD is within the control limit, the results are acceptable and are flagged as "certain matrix interference." If the average and RPD are both out of control, perform one more matrix spike and reevaluate the results.

10.6 If recoveries of both of the MS and MSD are outside the required range, check the recoveries of the LCS. If the LCS recoveries are reasonable, matrix interference is suspected; otherwise reanalyze the associated sample batch.

11. Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

12. References

U.S. EPA. 1996. Method 8260B, Revision 2, December.

U.S. EPA. 1996. Method 5035, December.

U.S. EPA. 1996. Method 5021, December.

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Appendix D. SOP O-3 Determination of Permanent Gases by GC/FID and GC/TCD

File Name PermGases SOP Ver.1.1
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O-3 Determination of Permanent Gases by GC/FID and GC/TCD

1. Scope and Application

1.1 This SOP describes the procedures used to analyze methane, acetylene, ethene, ethane, CO_2, and H_2S in aqueous solutions and gas phases by headspace GC/TCD/FID.

1.2 This method uses a headspace autosampler interfaced to a gas chromatograph equipped with a capillary column and thermal conductivity detector (TCD) in series with a flame ionization detector (FID).

1.3 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, and leachates; and gas phases in equilibrium with such aqueous solutions.

2. Summary of Method

2.1 Permanent gases are introduced to the chromatographic column using a Tekmar 7000 headspace autosampler equipped with a 0.1, 1.0, or 2.0-mL injection loop. The loop volume will be changed depending on the concentration of the gases in the samples.

2.2 The column is temperature programmed to separate the analytes, which are then identified and quantitated by FID and/or TCD using external standards.

2.3 The analysis time for each run is approximately 25 minutes.

3. Interferences

3.1 Method interferences may be caused by volatile contaminants within the laboratory, and volatile impurities in the reagent water, reagents, glassware, and other sample processing apparatus. Analyses of laboratory reagent blanks provide information about the presence of contaminants.

3.2 Interfering contamination may also occur when a sample containing low concentrations of analytes is analyzed immediately after a sample containing relatively high concentrations of the analytes. It is recommended that one or more laboratory reagent blanks be analyzed after the analysis of a highly contaminated sample to check for cross contamination.

4. Safety

Exposure to each chemical should be minimized. Pure standard and stock standard solutions of these analytes should be handled in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of the hydrogen sulfide.

5. Apparatus

5.1 GC/FID, TCD – Shimadzu 14A GC equipped with a Tekmar 7000HT Gas Chromatography Headspace Autosampler with carousel. The data acquisition and analysis system is the HP Chemstation version G2072AA installed on a Dell PC.

5.1.1 Column: Supelco Carboxen 1010 PLOT capillary column 30 m x 0.179mm.

5.1.2 Carrier gas: Helium at 22 mL/min.

5.1.3 Oven temperature program (total run time = 9.75 min.):

— Initial temperature 35 °C, hold 1 min
— Ramp at 35 °C/min to 225 °C, hold 1 min.

5.1.4 FID temperature: 200 °C.

5.1.5 TCD temperature: 230 °C.

5.1.6 TCE current: 125.

5.1.7 Injection port temperature: 230°C.

5.2 Tekmar headspace autosampler parameters:

5.2.1 Platen temperature: 50 °C.

5.2.2 Platen equilibrium time: 0 min.

5.2.3 Sample equilibrium time: 5 min.

5.2.4 Mix time: 5 min.

5.2.5 Mix power: 8.

5.2.6 Stabilize time: 1 min.

5.2.7 Pressure time: 0.2 min.

5.2.8 Loop time: 0.4 min.

5.2.9 Injection time: 1.00 min.

5.2.10 Pressure equilibrium time: 0.08 min.

5.2.11 Sample loop volume: 0.1, 1.0, or 2.0 mL.

5.2.12 Sample loop temperature: 180 °C.

5.2.13 Line temperature: 180 °C.

6. Reagents

6.1 Reagent water: deionized water, free of the analytes of interest. Water should be filtered through a 0.2 µm filter. At least one method blank should be performed for each batch of samples.

6.2 Methanol Optima grade (Fisher Scientific, Inc.).

6.3 Stock standard solutions: Gas stock standard, purchased from Scott Specialty Gases, Cat. # 2-3438, or other qualified vendors at 500 µg/mL, and Cat. # 2-3437 at 100 µg/mL, and Aldrich Chemical Company, Cat #29,544-2 at 99.5+%.

6.4 LCS standard: The LCS spike standard is made from a source other than that used for the preparation of the initial calibration standard.

6.5 Working standards: Due to the nature of preparing a calibration curve using permanent gases, there are no working standard solutions used.

Note: The maximum lifetime for gas standards in sealed 1-mL vials stored at 4 °C is 2 weeks or sooner if comparison with initial calibration curve or with check standards indicates a problem.

7. Sample Collection, Preservation and Storage

7.1 Twenty-mL microcosm headspace vials are used for permanent gas analysis following headspace analysis for volatile chlorinated organics.

7.2 Sample preservation and holding time for the analytes that can be determined by this method include cooling the samples to 4 °C ± 2 °C and holding them no longer than 72 hours prior to analysis.

8. Procedure

8.1 Operating Conditions

8.1.1 Make sure that all gas cylinders have a tank pressure of 300 psi or greater prior to starting the analysis. Change gas cylinders at or before the 300 psi gauge level.

8.1.2 Confirm that the carrier gas pressure (Carrier 1) through the column is 1 kg/cm^2 (14.2 psi, 9.8 kPa).

8.1.3 Confirm that the detector gas hydrogen pressure is set to 35 kPa (50 psi) and the air pressure is set to 55 kPa (80 psi). Adjust these settings as necessary.

8.1.4 Confirm that the flame is lit. Make sure the FID is zeroed.

8.1.5 Monitor the detector and injector temperatures and confirm that they are set to values listed in Section 5.1.

8.1.6 Confirm that the column initial temperature is 35 °C.

8.2 Initial Calibration

8.2.1 Initial calibration standards should be prepared over a range of concentrations expected in the sample or should define the working range of the detector. One of the standards should be at a concentration near, but above, the method detection limit.

8.2.2 Prepare a minimum of ten calibration standards for methane, ethane, ethene, and acetylene by injecting 0.05, 0.10, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, and 5.0 mL of the gas standard into a sealed headspace vial.

8.2.3 For the calibration of hydrogen sulfide on the TCD, prepare a minimum of eight calibration standards by injecting 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mL of pure H_2S gas into a sealed headspace vial.

8.2.4 For the calibration of carbon dioxide, prepare a minimum of seven standards by injecting 0.25, 0.50, 0.75, 2.0, 3.0, 3.5 and 5.0 mL of gas standard into a sealed headspace vial.

8.2.5 The highest or the lowest calibration point can be dropped for some compounds if the calibration linearity can not meet method requirement. The calibration standards should be loaded onto the autosampler immediately after preparation.

8.2.6 Standards and QC samples are placed into various positions in the auto injector, and these positions and sample identifications are logged into the sample analysis logbook and computer system.

8.2.7 Edit a sequence in the Chemstation software which corresponds to the sequence in the Tekmar 7000HT Headspace autosampler, (Method 4). Load

Method 4 to the memory and press 'start' to start the autosampler, and begin the analysis procedures.

8.2.8 Using known, quantitative volume injections of each calibration standard, tabulate peak area responses against the mass of each permanent gas injected. The Ideal Gas Law is used to convert volume of gas injected to mass of gas injected. The results are used to prepare a calibration curve for each analyte. During this procedure, retention times must be recorded. The Chemstation software will do this step automatically based on specified programming procedures. See the Chemstation software manual for details of these automated procedures.

8.2.9 Linearity. The correlation coefficient of the multipoint external calibration curve is used to evaluate the linearity and validity of the calibration curve for each analyte. The correlation coefficient of the calibration curve regression (r) must be greater than or equal to 0.99 for the calibration curve to pass QC.

8.3 Sample Analyses

8.3.1 Prepare standards, ICV, CCV and LCS samples.

8.3.2 Samples, standards, and QC samples are placed into various positions in the auto injector.

8.3.3 This sample order should allow for the initial analysis of a reagent blank, and a system calibration check sample prior to sample analysis. If required, recalibrate as described in Section 8.2.

8.3.4 Edit a sequence in the Chemstation program. Make sure the sequence in the headspace autosampler matches that in Chemstation. Log the vial positions and their sample identifications in the sample analysis logbook and computer system. Press "start" on the headspace autosampler to start the sequence.

8.3.5 The width of the retention time window is used to make identifications of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

8.4 Data Analysis. Calculation, and Reporting

8.4.1 An analyte is identified by comparison of a sample chromatogram with the standard chromatograms generated in developing the standard calibration

curve as discussed in Section 8.2. The retention time (RT) of an unknown peak is compared to the RTs of analytes within the standards to identify peaks within a sample chromatogram.

8.4.2 Elution of sample components (RT) must be within ± 5% of the RT of the standard component to verify identification of unknows within the sample chromatograms.

8.4.3 To obtain the correct peak areas, proper integration parameters should be used for standard and sample integration. The Chemstation auto integrate function should be used for the integration of all peaks.

8.4.4 Calculate concentration of each analyte, in µg/L, by first referring to the appropriate calibration curve to determine the mass of analyte, then dividing this mass by the sample loop volume in L. Alternatively, when the response is shown to be linear, use the following equation:

$$\mu g\ Analyte/L = [(A - b)/M]/V \quad (1)$$

where A = peak area; b = y-intercept of analyte calibration curve; M = slope of analyte calibration curve = peak area/mass of analyte; and V = analyte injection volume. All data including sample concentrations can be generated directly by using the Chemstation Chromatography Software.

8.4.5 Report results in µg/L for each analyte.

9. Quality Control

9.1 QC Samples

Method blank (MB), and laboratory control samples (LCS) should be analyzed on a daily basis or for each analytical batch (20 samples or less), whichever is more frequent.

9.2 QC Limits

9.2.1 Before any samples are analyzed, it should be demonstrated with a method blank, that the system is reasonably free of contamination that would interfere with the determination of any analytes of interest.

9.2.2 The initial calibration curve should have a correlation coefficient, r, of at least 0.99 with a minimum of three concentration points plus a blank. The control limit for initial calibration verification (ICV) is 90% - 110%.

9.2.3 Perform the daily or continuing calibration verification (CCV) by measuring the midpoint calibration concentration before analysis of every 10 samples. The control limit for the CCV is 85%-115%. A reagent blank should be analyzed prior to each CCV sample.

9.2.4 The analyte retention times in the CCV sample must also be evaluated immediately after or during data acquisition. If the retention time for any analyte changes by more than three standard deviations of the retention time observed in generating the calibration curve, the chromatographic system must be inspected for malfunctions and corrections must be made, as required.

9.2.5 The recoveries of the analytes in the LCS should be within 80%-120%.

9.2.6 All calibration and method blanks should have concentrations of analytes less than the practical quantitation limit (PQL).

9.3 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL (vol%)	MDL (ppmv)	Date	PQL (vol%)	PQL (ppmv)	Source
Methane	$9.0x10^{-4}$	9.0	7/24/01	$1.5x10^{-3}$	15.0	UWRL
Acetylene	$5.5x10^{-4}$	5.5	7/24/01	$9.2x10^{-4}$	9.2	UWRL
Ethene	$5.1x10^{-4}$	5.1	7/24/01	$8.6x10^{-4}$	8.6	UWRL
Ethane	$4.8x10^{-4}$	4.8	7/24/01	$8.0x10^{-4}$	8.0	UWRL
CO_2	0.225	2,250	8/2/01	0.376	3,760	UWRL
H_2S	0.052	515	7/24/01	0.086	859	UWRL

10.0 Corrective Action

10.1 If the method blank (or instrument blank) indicates a result higher than the PQL, the containers, reagents, and analytical system should be carefully examined and cleaned until the background disappears before samples can be analyzed.

10.2 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

10.3 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

11. Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

12. References

Supelco. 1996. *Supelco Application Note 112*. Sigma-Aldrich Co., Bellefontae, PA.

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Appendix E. SOP I-1 Determination of Inorganic Anions by IC Method: EPA 300.0

File Name IC SOP Ver. 1.1 7/2001
Version No 1.1
Revision Date 7/2001

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I-1 Determination of Inorganic Anions by IC; Method: EPA 300.0

1. Scope and Application

1.1 This method covers the determination of the following inorganic anions: chloride, nitrate-N, nitrite-N, sulfate.

1.2 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, aqueous extracts of solids, and leachates (when no acetic acid is used).

2. Summary of Method

2.1 A small volume (25 µL) of subsample from a 5 mL sample is automatically introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, separator column, suppressor device, and conductivity detector.

2.2 In order to use this method for solids an extraction procedure must be performed.

3. Interferences

3.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or spiking can be used to solve most interference problems.

3.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus; that lead to discrete artifacts or elevated baseline in ion chromatograms.

3.3 Samples that contain particles larger than 0.45 µm and reagent solutions that contain particles larger than 0.20 µm require filtration to prevent damage to instrument columns and flow systems.

4. Apparatus

4.1 Ion chromatograph (DX-500) - Analytical system complete with ion chromatograph and all required accessories , analytical columns, compressed gasses and detectors.

4.1.1 Anion guard column: AG4A-SC (4 mm) P/N 043175

4.1.2 Anion separator column: Dionex AS4A-Sc 4 mm column, P/N 043174

4.1.3 Anion suppressor device: Anion micromembrane suppressor-II ASRS-Ultra 4 mm, P/N 053496

4.1.4 Detector: Conductivity cell, CD20 cell, P/N 044130

4.2 The Dionex PeakNet Data Chromatography Software (Version 2.0) was used to generate all data.

4.3 Dionex Automated sampler.

5. Reagents

5.1 Reagent water: Deionized water, free of the anions of interest. Water should be filtered through a 0.2 µm filter.

5.2 Eluent solutions: Sodium bicarbonate 1.7 mM, sodium carbonate 1.8 mM. Dissolve 0.2856 g sodium bicarbonate ($NaHCO_3$) and 0.3816 g of sodium carbonate (Na_2CO_3) in reagent water and dilute to 2 L with reagent water.

5.3 Stock standard solutions, 1,000 mg/L; Stock standard solutions may be purchased as certified solutions or prepared from ACS reagent grade materials (dried at 105° C for 30 min.) as listed below.

5.3.1 Chloride(Cl^-) 1,000 mg/L: Dissolve 1.6485 g sodium chloride (NaCl) in reagent water and dilute to 1 L with reagent water.

5.3.2 Nitrate(NO_3-N) 1,000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$) in reagent water and dilute to 1 L.

5.3.3 Nitrite (NO_2-N) 1,000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaNO_2$) in reagent water and dilute to 1 L.

5.3.4 Phosphate (PO_4^{3-}-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate, monobasic (KH_2PO_4) in reagent water and dilute to 1 L.

5.3.5 Sulfate (SO_4^{2-}) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4) in reagent water and dilute to 1 L.

Note: Stability of standards: Stock standards are stable for at least 1 month when stored at 4° C. Dilute working standards should be prepared weekly, except those that contain nitrite and phosphate should be prepared fresh daily.

6. Sample Collection, Preservation and storage

6.1 Samples should be collected in scrupulously clean containers. Do not clean containers with strong acids or detergents because they leave traces of ions on the container walls; these ions may interfere with analysis.

6.2 Sample preservation and holding times for the anions that can be determined by this method are as follow:

Analyte	Preservation	Holding time
Chloride	Not required	28 days
Nitrate-N	cool to 4 C	48 hours
Nitrite-N	cool to 4 C	48 hours
Sulfate	cool to 4 C	28 days

The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment. It is recommended that all samples be cooled to 4° C and held for no longer than 48 hours for this method.

7. Procedure

7.1 Operating Conditions

7.1.1. Turn the system power on and set the control button on the system panel to Local. Turn on the system helium and confirm that the pressure is between 62 MPa (90 psi) and 76 MPa (110 psi).

7.1.2 Confirm that the eluent and H_2O containers are full.

7.1.3 Ensure that the pump flow rate adjustment is correct, and turn on the pump. (Eluent flow rate 2.0 mL/min).

7.1.4 Set the detector range to the appropriate operating range (typically 30 µS).

7.1.5 Verify the sample loop volume is 25 µL.

7.1.6 A stable base line indicates equilibrium conditions. Adjust detector offset to zero out eluent conductivity; with the fiber or membrane suppressor adjust the regeneration flow rate to maintain stability.

7.2 Calibration

7.2.1 For each analyte of interest, prepare calibration standards at a minimum of three concentration levels and a blank by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with reagent water. If the working range exceeds the linear range of the system, samples should be diluted. One of the standards should be at a concentration near, but above, the method detection limit if the system is operated on various attenuator ranges. The other standards should correspond to the range of concentrations expected in the sample or should define the working range of the detector. Unless the attenuator range settings are proven to be linear, each range must be calibrated individually.

7.2.2 Using injections of 5.0 mL of each calibration standard, tabulate peak area responses against the concentration. The results are used to prepare a calibration curve for each analyte. During this procedure, retention times must be recorded.

7.2.3 The calibration curve must be verified on each working day, or whenever the anion eluent is changed, and after every 20 samples. If the response or retention time for any analyte varies from the expected values by more than ± 10%, the test must be repeated, using fresh calibration standards. If the results are still more than ± 10%, a new calibration curve must be prepared for that analyte.

7.2.4 Nonlinear response can result when the separator column capacity is exceeded (overloading). The response of the detector to the sample when diluted 1 to 1 and when not diluted should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

7.3 Sample Pretreatment

7.3.1 Samples should be filtered through 0.45 µm filters before injection if they were not filtered after collection.

7.3.2 If samples may contain high concentrations of organic constituents, filter them through Dionex OnGuard P syringe filters before injection. Be sure to activate the cartridge use by pushing 5 mL of deionized water through the filter.

7.3.3 Dilution: The concentrations of analytes can vary widely from sample to sample, so no one dilution can be recommended for all samples of one type. However, some samples can be analyzed with no dilution. The

analyte concentrations in many water samples are low enough so that dilution is usually not necessary.

The solution used to dilute the sample (diluant) may be deionized water, but eluent is preferable since diluting in eluent minimizes the effect of the water dip at the beginning of the chromatogram. If eluent is used as the diluant, then eluent should also be used as diluant to prepare the calibration standard. This is most important for chloride which elutes near the water dip.

7.4 Sample Analyses

7.4.1 Check system calibration daily and if required, recalibrate as described in Section 7.2.

7.4.2 Load and inject a fixed amount of well mixed sample. Flush injection loop thoroughly, using each new sample. Use the same size loop for standards and samples.

7.4.3 The width of the retention time window used to make identifications of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

7.4.4 If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

7.4.5 If the resulting chromatogram fails to produce a adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

7.5 Data Analysis. Calculation, and Reporting

7.5.1 Calculate concentration of each anion, in mg/L, by referring to the appropriate calibration curve . Alternatively, when the response is shown to be linear, use the following equation:

$$C = H \times F \times D \qquad (1)$$

where C = mg anion/L; H = peak area; F = response factor = concentration of standard/height (or area) of standard; and D = dilution factor for those samples requiring dilution.

In fact, all data including sample concentrations can be generated directly by using the Dionex PeakNet Data Chromatography Software.

7.5.2 Report results in mg/L.

7.5.3 Report

7.5.3.1 $N0_2$- as N

7.5.3.2 $N0_3$- as N

7.5.3.3 $P0_4^{3-}$ as P

8. Quality Control

8.1 QC Samples

Method blank (MB), matrix spike (MS), matrix spike duplicate (MSD), and laboratory control samples (LCS) should be analyzed on a daily basis or each analytical batch (20 samples or less).

8.2 QC Limits

8.2.1 Before any samples are analyzed, it should be demonstrated with a method blank, that the system is reasonably free of contamination that would interfere with the determination of any analytes of interest.

8.2.2 Initial calibration curve should have a correlation coefficient, r, of at least 0.995 with a minimum of three concentration points plus a blank. The control limit for initial calibration verification (ICV) is 90% - 110%.

8.2.3 Perform the daily or continuing calibration verification (CCV) by measuring the midpoint calibration before sample analysis of every 10 samples. The control limit for the CCV is 95%-105%.

8.2.4 The recoveries of the analytes in MS, MSD, and LCS should be within the following control limits: the control limit of lab control samples is 80%-120%; and the control limit for the matrix spikes is 75%-125%.

8.2.5 All calibration and method blanks should have concentrations of analytes less than the practical quantitation limit (PQL).

8.3 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL (mg/L)	Date	PQL (mg/L)	Source
Chloride	0.03	7/10/01	0.06	UWRL
Nitrate-N	0.02	7/10/01	0.04	UWRL
Nitrite-N	0.02	7/10/01	0.03	UWRL
Sulfate	0.10	7/10/01	0.17	UWRL

9.0 Corrective Action

9.1 If the method blank (or instrument blank) indicates a result higher than the PQL, the containers, reagents, and analytical system should be carefully examined and cleaned until the background disappears before samples can be analyzed.

9.2 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

9.3 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

9.4 If there is not enough sample for a MSD or even for a MS, the LCS/LCSD may be used for the QC report.

9.5 If one of the recoveries of the MS and MSD is outside of the control limit check the average of the MS and MSD. If the average is also out of control, perform one more matrix spike.

9.6 If recoveries of both of the MS and MSD are outside the required range, check the recoveries of the LCS. If the LCS recoveries are reasonable, matrix interference is suspected and should be controlled as discussed in Section 3; otherwise reanalyze the associated sample batch.

10. Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

11. References

EPA Method 300.0, Revision 2.1, 1993.

EQL - UWRL

8200 Old Main Hill, Logan, UT 84322-8200

(435)797-3155; (435)797-3663, fax.

Appendix F. SOP I-2 Determination of Total and Dissolved Organic Carbon
Method: EPA 415.1

File Name TOC/DOC SOP Ver. 1.0 7/2001

Version No 1.0

Revision Date 7/2001

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I-2 Determination of Total and Dissolved Organic Carbon Method: EPA 415.1

1. Scope and Application

1.1 This method includes the measurement of total and dissolved organic carbon in drinking water, surface water, groundwater and saline waters, and domestic and industrial wastewaters.

1.2 The method is most applicable to measurement of organic carbon above 1 mg/L.

2. Summary of Method

2.1 Sample introduction is facilitated by liquid reagent flow and pressurized gas which delivers the sample to a UV reactor for wet chemical oxidation. The CO_2 that is formed is measured by nondispersive IR detection. The amount of CO_2 in a sample is directly proportional to the concentration of carbonaceous material in the sample.

2.2 The sample is pre-treated with phosphoric acid to covert inorganic C (carbonate and bicarbonate ions) to CO_2. The sample is then bubbled with N_2 gas to remove this CO_2 generated from inorganic carbon sources so the measurement can be made without inorganic carbon interference.

2.3 Dissolved organic carbon measurements can be made by filtering a sample through a 0.45 µm filter then acidifying and bubbling the sample as described in Section 2.2, prior to determing organic carbon of the filtrate.

2.4 Sample can also be analyzed for total C if samples are injected into the instrument without removal of the inorganic carbon. Inorganic carbon can then be determined by difference.

3. Sample Handling and Preservation

3.1 Collect and store samples in amber glass bottles with TFE-lined caps.

3.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the holding time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4° C) and protected from sunlight and atmospheric oxygen.

3.3 In instances where analysis cannot be performed within 2 hours from the time of sampling, the sample is acidified to a pH _ 2 with phosphoric acid and bubbled with N_2 for a minmum of 10 minutes to remove inorganic carbon and preserve the sample for later analysis.

4. Interferences

4.1 Carbonate and bicarbonate carbon represent an interference under the conditions of this test and must be removed or accounted for in the final calculation. Removal of carbonate and bicarbonate by acidification and purging with nitrogen can result in the loss of volatile organic substances.

4.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe. The openings of the syringe limit the maximum size of particles which may be included in the sample.

5. Apparatus

5.1 Total organic carbon analyzer: DC-180 (Dohrmann).

6. Reagents

6.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double-distilled water is recommended.

6.2 Organic carbon stock solution. Weigh out 425 mg of reagent grade potassium hydrogen phthalate, $C_8H_5O_4K$, KHP, dried to a constant weight. Thoroughly transfer the KHP to a 100 mL volumetric flask and dissolve with 50 to 75 mL of reagent water. Add 0.1 mL of reagent grade concentrated phosphoric acid and dilute to the 100 mL mark with reagent water. Store solution in dark glass under refrigeration; 1.00 mL = 2.00 mg carbon. For working standards, dilute this stock solution to a concentration appropriate for a given analysis; usually to a working concentration of 20 to 200 mg C/L.

6.3 Inorganic carbon stock solution. Dissolve 0.3532 g anhydrous sodium carbonate, Na_2CO_3, dried to constant weight, in 50 to 75 mL reagent water, and dilute to 100 mL; 1.00 mL = 1.00 mg carbon.

6.4 Potassium persulfate reagent, 2%. Prepare by dissolving 20 g of reagent grade potassium persulfate ($K_2S_2O_8$) in 1 L of reagent water. Add 2 mL of concentrated phosphoric acid and mix well. These quantities need not be measured with great accuracy; ± 5% is sufficient. The desired pH is 3. Store in cool dark place.

6.5 Phosphoric acid reagent, 2%. Dilute 20 mL reagent grade 85% phosphoric acid to 100 mL with reagent grade water.

6.6 Carrier gas. Purified oxygen, CO_2-free, containing less than 1 ppm hydrocarbon.

Note: Stability of standards: Stock standards are stable for 1 month when stored at 4° C. Dilute working standards should be prepared weekly.

7. Procedure

Follow manufacturer's instructions for analyzer testing, calibration and operation.

7.1 Before starting check the following:

- 7.1.1 Distilled water bottle should be full.
- 7.1.2 Persulfate reagent bottle should be full.
- 7.1.3 Waste receiving bottle should be empty.

7.2 Operating Conditions

- 7.1.1 Turn on O_2 tank and verify that the cylinder gas pressure is set at 30 psi. Turn on the printer.
- 7.1.2 Turn on power by pressing large white button on face of instrument.
- 7.1.3 Press "1" on the keyboard on the face of the instrument to turn the system on.
- 7.1.4 Press "1" on the keyboard on the face of the instrument again to turn the O_2, UV lamp, and pump on.
- 7.1.5 Press "+/-" on the keyboard on the face of the instrument to return to the main menu.
- 7.1.6 Check that all tubing within the UV reaction chamber on the left side of the instrument, and in mixing chamber on the right side of the instrument is connected.

7.1.7 Move tubes on the peristaltic pump within the mixing chamber to upper tier of the pump, making sure the tubing seats securely.

7.1.8 Verify the the carrier gas pressure contoller gauge on the right side of the instrument is set to 5 psi.

7.1.9 Press "5" on the keyboard on the face of the instrument to monitor the baseline. Within 10 to 15 minutes, the baseline reading should drop to 6 to 8 ppm CO_2.

7.2 Calibration

7.2.1 Prepare a calibration standard according to Step 6.2 above. The instrument is calibrated against a single point standard typically at 20 or 200 mg C/L.

7.2.2 While waiting for baseline to drop, check that:

7.2.2.1 Analysis mode is set to "Syringe inject" (Menu sequence = 2,3,7).

7.2.2.2 Calibration mode is set to "Syringe calibration" (Menu sequence = 2,4,4).

7.2.3 Rinse a 5 mL syringe three times with distilled water.

7.2.4 Rinse the syringe three times with the standard.

7.2.5 Draw the standard to the 1 mL on the syringe.

7.2.6 Press "Run" on the keyboard on the face of the instrument.

7.2.7 Enter "1000" for syringe volume (1 mL).

7.2.8 At "Inject now!" screen prompt, inject 1 mL of a standard into the injection port on the front of the instrument.

7.2.9 At the "Continue?" prompt, press "No" on the keyboard on the face of the instrument.

7.2.10 Press "+/-" on the keyboard on the face of the instrument to return to the main menu.

7.2.11. Press "2" on the keyboard on the face of the instrument to enter the set-up menu.

7.2.12 Press "4" on the keyboard on the face of the instrument to enter the calibration modes menu.

7.2.13 Press "5" on the keyboard on the face of the instrument to update the calibration factor. The calibration factor should read 7.8×10^{-2}.

7.2.14 At the "Enter std concentration" screen prompt, enter the concentration of the standard that was just run. Record the calibration factor that is displayed.

7.2.15 Press "+/-" on the keyboard on the face of the instrument to return to the main menu to prepare to analyze a sample.

7.3 Sample Pretreatment

7.3.1 Samples to be analyzed for dissolved organic carbon (DOC) should be filtered through a 0.45 µm filter before injection if they were not filtered after collection.

7.3.2 Dilution: The concentrations of analytes can vary widely from sample to sample, so no one dilution can be recommended for all samples of one type. However, some samples can be analyzed with no dilution. The analyte concentrations in many water samples are low enough so that dilution is usually not necessary. Dilution must be provided, however, if the sample concentration exceeds the standard used to calibrate the instrument. Dilution should be carried out using distilled water to reduce the sample organic carbon concentration to within the concentration range of the standard used for instrument calibration. Note that the maximum concentration that can be measured by the instrument is 200 mg C/L.

7.4 Sample Analyses

7.4.1 Recalibrate the instrument daily as described in Section 7.2.

7.4.2 Rinse a 5 mL syringe with distilled water three times.

7.4.3 Rinse the syringe with sample three times, wasting the rinsate into a small beaker.

7.4.4 Draw the sample to the 1 mL mark in the syringe in preparation for injection into the instrument.

7.4.5 When the instrument is at the main menu after calibration press "Run" on the keyboard on the face of the instrument.

7.4.6 Enter "1000" on the keyboard for syringe volume (1 mL).

7.4.7 At the "Inject now!" screen prompt, inject 1 mL of a sample via the injection port on the front of the instrument.

7.4.8 At the "Continue?" screen prompt, press "Yes" on the keyboard on the face of the instrument and repeat Steps 7.4.2 through 7.4.8 until all samples are analyzed.

7.5 System Shutdown

7.5.1 At the "Continue?" screen prompt, press "No"on the keyboard on the face of the instrument.

7.5.2 Press "+/-" on the keyboard on the face of the instrument to return to the main menu.

7.5.3 Press "1" on the keyboard on the face of the instrument to turn the system off.

7.5.4 Press "2" on the keyboard on the face of the instrument to turn all components off.

7.5.5 Press "+/-" on the keyboard on the face of the instrument to return to the main menu.

7.5.6 Turn the O_2 tank and printer off.

7.5.7 Move the tubes on the peristaltic pump within the mixing chamber to the lower tier of the pump, and disconnect the tubing from the top of the front of the UV chamber to depressurize the chamber.

7.5.8 Turn off the power by pressing the large white button on the face of the instrument.

7.6 Data Analysis, Calculation, and Reporting

7.6.1 The instrument readout is in mg C/L based on the single point calibration standard used in the calibration procdure described in Section 7.2.

7.6.2 Report results in mg C/L.

8. Quality Control

8.1 QC Samples

8.1.1 Method blank (MB), matrix spike (MS), matrix spike duplicate (MSD), and laboratory control samples (LCS) should be analyzed on a daily basis or each analytical batch (20 samples or less). The LCS for this analyte will be Logan Tap Water collected in the EQL at the UWRL.

8.1.2 Continuing calibration verification (CCV) samples should be run at least every 10 samples and at the end of a run.

8.2 QC Limits

8.2.1 Before any samples are analyzed, it should be demonstrated with a method blank, that the system is reasonably free of contamination that would interfere with the determination of any analytes of interest.

8.2.2 The initial calibration verification (ICV) sample should be 90% - 110% of the expected concentration.

8.2.3 The CCV sample should be run after the analysis of 10 samples. The control limit for the CCV is 90%-110% of the expected value.

8.2.4 The recoveries of the analytes in MS, MSD, and LCS should be within the following control limits: for the LCS 90%-110% of the expected value; and for the matrix spikes 75%-125% of the expected value.

8.2.5 All blanks should have concentrations of analytes less than the practical quantitation limit (PQL).

8.3 Method Detection Limit (MDL)

MDL = 0.12 mg/L; UWRL determined 6/20/01.

8.4 Practical Quantitation Limit (PQL)

PQL = 0.2 mg/L; UWRL determined 6/20/01.

9.0 Corrective Action

9.1 If the method blank (or instrument blank) indicates a result higher than the PQL, the containers, reagents, and analytical system should be carefully examined and cleaned until the background disappears before samples can be analyzed.

9.2 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

9.3 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

9.4 If there is not enough sample for a MSD or even for a MS, the LCS/LCSD may be used for the QC report.

9.5 If one of the recoveries of the MS and MSD is outside of the control limit check the average of the MS and MSD. If the average is also out of control, perform one more matrix spike.

9.6 If recoveries of both of the MS and MSD are outside the required range, check the recoveries of the LCS. If the LCS recoveries are reasonable, matrix interference is suspected and should be controlled as discussed in Section 4; otherwise reanalyze the associated sample batch.

10. Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

13. References

EPA 415.1.

EQL - UWRL
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Appendix G. SOP I-7 Determination of pH, ORP, and Electrical Conductivity

File Name 1-07 Probes SOP Ver. 1.1
Version No. 1.1
Revision Date 10/2001

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I-7 Determination of pH, ORP, and Electrical Coductivity

1.0 Scope and Application

1.1 This method covers the determination of pH, ORP, and electrical conductivity using various probe sensing methods.

1.2 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, extracts of solids, and leachates. Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high ionic strength (> 0.2 M) solutions.

2.0 Summary of Method

2.1 *Electrometric pH measurement.* Electrometric pH measurement is carried out by determination of the activity of hydrogen ions in a sample via potentiometric measurement using a glass electrode and a reference electrode. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by interpolation.

2.2 *ORP.* Redox potential, or oxidation/reduction potential (ORP) is measured electrometrically with a $Ag/AgCl_2$ electrode using a saturated KCl reference electrode. ORP readings should be adjusted to reference the hydrogen electrode by adding 235.8 mV to the measured ORP reading.

2.3 *Electrical Conductivity.* The electrical conductivity of a sample is a numerical expression of the ability of aqueous solutions to carry an electric current. This ability depends on the presence of ions, their total concentration, their mobility, valence, and relative concentrations, and on the temperature at which the measurement is determined. The substantial temperature dependence is usually expressed as a percent/^{o}C at 25 ^{o}C. Electrical conductivity measurements are made using a laboratory conductivity meter with a temperature-compensating probe.

3.0 Interferences

3.1 *pH.*

3.1.1 The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH > 10. Reduce this error by using special "low sodium error" electrodes.

3.1.2 pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the slope of emf versus pH increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures. *Always report temperature at which pH is measured.*

3.2 *ORP.*

3.2.1 ORP measurements may not be accurate since most groundwater environments are not at redox equilibrium (Lindberg and Runnells, 1984).

3.2.2 Several potentially important redox couples (e.g., O_2/H_2O; NO_3^-/N_2aq; SO_4^{2-}/H_2S; CH_4aq/HCO_3^-) do not react rapidly and reversibly at the platinum surface, and are therefore, not measured by the platinum electrode (Drever, 1988).

3.2.3 The ORP of waters in which the iron (Fe_2^+/Fe_3^+) redox couple dominates is among those most accurately measured by the platinum electrode.

3.3 *Electrical Conductivity.*

3.3.1 General biological or chemical fouling on the conductivity electrode can lead to erratic or slowly responding readings. Electrical conductivity electrodes should be cleaned using procedures stated in the operating manual if such deteriorated performance is evident from routine calibration checks.

3.3.2 Electrical conductivity measurements are highly sensitive to temperature, and although this is not strictly an interference issue, significant errors can result from inaccurate temperature measurement. Temperature measurements accurate to ± 0.1°C are necessary to provide reliable electrical conductivity data.

4.0 Apparatus

4.1 *pH.*

4.1.1 ORION low maintenance triode.

4.1.2 Model 250A or Model 290A portable pH/ISE meters.

4.1.3 pH 4.0 and 7.0 buffer solutions.

4.1.4 150 mL glass beakers.

4.2 *ORP.*

4.2.1 Corning Platinum Redox Combination cell.

4.2.2 Model 250A or Model 290A portable pH/ISE meters.

4.3 *Electrical Conductivity.*

4.3.1 ACCUMET Conductivity Meter, Model 30.

4.3.2 ACCUMET 13-620-160 Conductivity Cell.

5.0 Reagents

5.1 *pH.*

5.1.1 Saturated KCl solution.

5.1.2 pH 4.0 and 7.0 buffer solutions.

5.1.3 Deionized water.

5.2 *ORP.*

5.2.1 Standard redox solutions.

Standard Solution	Potentials of Pt Electrode versus Selected Reference Electrode KCl Saturated at 25 °C	Weight of Chemicals Needed/L Aqueous Solution at 25°C
Light's Solution	+476	39.21 g ferrous ammonium sulfate 48.22 g ferric ammonium sulfate 56.2 mL sulfuric acid, sp gr 1.84
ZoBell's Solution*	+229	1.4080 g potassium ferrocyanide 1.975 g potassium ferricyanide 7.4555 g potassium chloride

* Store in dark plastic bottle in a refrigerator.

5.2.2 Saturated KCl fill solution.

5.2.3 Deionized water.

5.3 *Electrical Conductivity.*

5.3.1 0.01 M KCl solution.

5.3.1.1 Dry 1.00 g KCl overnight in oven at 103° C.

5.3.1.2 Boil 1.5 L of deionized water and let cool to room temperature 25° C.

5.3.1.3 Measure 0.7456 g of dried KCl and mix it with the cooled distilled water.

5.3.1.4 Dilute to 1 L at 25° C.

5.3.2 Deionized water.

6.0 Sample Collection, Preservation and Storage

6.1 *pH.* Samples will be collected in 50 mL test tubes as the filtrate from the processing of the 125-mL reactors. These samples should be analyzed as soon as possible after filtration of the 125-mL reactors. The holding time for these samples is 2 hours. The pH analyses should be run after EC measurements and before ORP measurements to minimize potential contamination during sample handling and analysis.

6.2 *ORP.* Samples for ORP analysis are the same samples as those for which pH is measured. Because of potential changes in ORP as samples are exposed to the ambient atmosphere, samples should be analyzed as soon as possible after filtration of the 125-mL reactors. During the analysis process, test tubes containing these samples should be covered with Parafilm™ to minimize oxygen diffusion into them.

6.3 *Electrical Conductivity.* Samples for EC analysis are the same samples as those for which pH and ORP are measured. These samples should be analyzed as soon as possible after filtration of the 125-mL reactors, and should be analyzed before pH to minimize potential contamination during sampling, handling and analysis.

7.0 Procedure

7.1 Daily Calibration.

7.1.1 *pH.*

7.1.1.1 Fill two beakers with a small amount of pH 4.0 and 7.0 buffers.

7.1.1.2 Press the **mode** key until the pH mode indicator is displayed.

7.1.1.3 Press **2nd cal.** key. After a few seconds **P1** will be displayed in the lower field indicating the meter is ready for the first buffer.

7.1.1.4 Rinse the electrode with distilled water, collecting the rinsate in a waste beaker. Blot dry the electrode with a soft tissue, and place it into the beaker with pH 4.0 buffer solution.

7.1.1.5 Press **Yes** once meter flashes **Ready**.

7.1.1.6 The **P2** prompt is then displayed for the second buffer. At this prompt repeat step 7.1.1.4 with the pH 7.0 buffer solution.

7.1.1.7 Press **Yes** once the meter flashes **Ready**.

7.1.1.8 Once calibrated with two buffers, press **Measure**.

7.1.1.9 Record the slope reading for the daily calibration on the Daily pH Calibration sheet.

7.1.2 *ORP.*

7.1.2.1 If the Eh probe has not been used for sometime let it soak in nitric acid for 10 minutes prior to use. Rinse it well with distilled water before calibration.

7.1.2.2 Run a daily probe check sample at the beginning of each sampling day using one of the redox standard solutions listed in Section 5.2.1.

7.1.2.3 Press the **Mode** key until the **mV** mode indicator is displayed.

7.1.2.4 Rinse the electrode with deionized water, collecting the rinsate in a waste beaker. Blot the electrode dry with a soft tissue, and then place the electrode into a beaker with the redox solution.

7.1.2.5 Press **Measure**.

7.1.2.6 Wait for a stable reading of mV. The reading should be within ± 10 mV of the given values of potentials listed in Section 5.2.1.

7.1.2.7 Record the calibration reading for the ORP calibration check solution on the Daily ORP Calibration sheet and on the data sheet for the samples being analyzed.

7.1.3 *Electrical Conductivy.*

7.1.3.1 Press **Setup** twice to view Setup 3. Press 2 to set the conductivity cell. Press 2 again to set the conductivity cell on 1.00/cm. Press **Enter**.

7.1.3.2 Press 4, on Setup 3, to set the temperature coefficient. Press 3 to set the temperature coefficient to 25° C. Press **Enter**. The screen should read that the **Set Temperature Coefficient is 2.00%/°C**. Press **Enter**.

7.1.3.3 Rinse the electrode well with deionized water to clean off salt deposits.

7.1.3.4 Place the electrode into the 0.01 M KCl solution.

7.1.3.5 Press **Standardize**. The meter should read 1413 µS/cm, if it does not, manually enter this value. Press **Enter**.

7.1.3.6 Press **Slope**, and record the cell constant on the EC Daily Calibration sheet.

7.2 Sample Analysis

7.2.1 *pH.*

7.2.1.1 Rinse the electrode with deionized water, collecting the rinsate in a waste beaker. Blot the electrode dry with a soft tissue, and place it into a beaker containing a sample to analyze.

7.2.1.2 Press **Measure** and wait for the **Ready** light to take a reading.

7.2.1.3 Record, on the datasheet provided, the pH and temperature displayed on the meter.

7.2.1.4 Rinse the electrode with distilled water, collecting the rinsate in a waste beaker. Blot the electrode dry with a soft tissue before placing it into the next sample.

7.2.2 *ORP.*

7.2.2.1 Rinse the Eh electrode with distilled water, collecting the rinsate in a waste beaker. Blot the electrode dry with a soft tissue, and place it into the sample.

7.2.2.2 When the reading stabilizes press **Measure**, wait for the **Reeady** light to take a reading, and record on the data sheet provided the mV reading and the temperature displayed on the meter.

7.2.2.3 Rinse the electrode with distilled water, collecting the rinsate in a waste beaker. Blot the electrode dry with a soft tissue before placing it into the next sample.

7.2.3 *Electrical Conductivity.*

7.2.3.1 Rinse the electrode with deionized water after calibration.

7.2.3.2 Move the electrode up and down gently in the sample until it reaches a stable reading on the meter display.

7.2.3.3 Record the conductivity directly from the meter display on to the EC Datasheet. Note the scale of the meter when recording the data on the datasheet, i.e., mS/cm or µS/cm.

7.2.3.4 Rinse the electrode with deionized water before placing it into the next sample.

7.3 Care for the Electrodes for Short-Term and Long-Term Storage

7.3.1 *pH.*

7.3.1.1 Rinse the electrode with deionized water after each use.

7.3.1.2 For short-term storage, soak the electrode in a pH 7.0 buffer solution, and cover.

7.3.1.3 For long-term storage, soak the electrode in a pH 7.0 buffer solution to which 1.0 g of saturated KCl has been added, and cover. Confirm proper maintenance in the Maintenance Log.

7.3.2 *ORP.*

7.3.2.1 Cover the side hole to prevent drying of the electrode's KCl fill solution.

7.3.2.2 Check the level of the KCl fill solution in the probe daily and replace the solution monthly or more frequently as necessary.

7.3.2.3 Immerse the electrode in KCl fill solution when not in use.

7.3.2.4 Keep the electrode clean by rinsing off built up salts with deionized water. Confirm proper maintenance in the Maintenance Log.

7.3.3 *Electrical Conductivity.*

7.3.3.1 Rinse the electrode with distilled water after each use.

7.3.3.2 At the end of each microcosm batch (i.e. each sampling day) rinse the cell with deionized water.

7.3.3.3 Immerse the electrode and store it in deionized water when not in use. Confirm proper maintenance in the Maintenance Log.

8.0 Quality Control

8.1 QC Samples

An initial calibration verification (ICV), two continuing calibration verification (CCV), one laboratory control sample (LCS), a procedural blank (PB), and sample duplicates (SD) should be analyzed for each treatment batch (20 samples or less).

8.2 QC Limits

Before any samples are analyzed, it should be demonstrated with an ICV, that the probes are properly calibrated or are reading check solutions as they should.

9.0 Corrective Action

9.1 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

9.2 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

10.0 Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

11.0 References

American Public Health Association, American Water Works Association, Water Environment Federation. 1992. *Standard Methods for the Examination of Water and Wastewater*. 18th Edition. Clesceri, L.S., Eaton A.D., Greenberg, A.E. ORP. pp. 2-60-2-63.

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Appendix H. SOP I-6 Determination of Ferrous Iron using Ferrozine

File Name 1-06 Ferrozine SOP Ver. 1.1
Version No. 1.1
Revision Date 7/2001

Prepared/ Revised by:

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Approval:

Darwin L. Sorensen, Ph. D.
QA Officer

R. Ryan Dupont
Division Head, Environmental Engineering

I-6 Determination of Ferrous Iron using Ferrozine

1.0 Scope and Application

1.1 This method covers the determination of ferrous iron (Fe^{2+}).

1.2 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, extracts of solids, and leachates.

2.0 Summary of Method

2.1 Ferrozine forms a violet colored complex with ferrous iron and not with ferric iron. The intensity of the colored complexed formed is measured against standard solutions containing ferrous iron using a spectrophotometer at 562 nm. The relationship between absorbance and concentration follows Beer's Law. The linear range of the analysis is 0.1 to 10 mg/L ferrous iron.

2.2 In order to use this method for solids an extraction procedure must be performed.

3.0 Interferences

3.1 Ferrous iron is the reduced form of iron. Samples must be protected from exposure from oxygen or other oxidizing agents during collection, storage and analysis.

3.2 Samples should be analyzed immediately after collection. Analyses may need to be conducted in a glove box with a nitrogen-hydrogen atmosphere to completely exclude oxygen.

4.0 Apparatus

4.1 A spectrophotometer capable of measurements using a wavelength of 562 nm.

5.0 Reagents

5.1 Ferrozine reagent.

5.2 Hepes buffer.

5.3 NaOH.

5.4 HCl.

5.5 Ferrous ammonium sulfate.

6.0 Sample Collection, Preservation and Storage

6.1 Samples should be collected in scrupulously clean containers that have been rinsed with 50% nitric acid and thoroughly rinsed with deionized water.

6.2 Since ferrous iron is the reduced form of iron, all contact with oxidizing agents, including oxygen, will cause the conversion of Fe(II) to Fe(III). Samples must be analyzed immediately.

7.0 Procedure

7.1 Ferrozine reagent in 50 mM Hepes buffer: Dissolve 0.1 g ferrozine and 1.19 g Hepes in approximately 90 mL deionized water. Adjust pH to 7 with NaOH, dilute to 100 mL.

7.2 Ferrous iron standards: Prepare a 1,000 mg/L ferrous iron standard from ferrous ammonium sulfate in 0.005 M HCl. Weigh out 3.5139 g of ferrous ammonium sulfate and dilute to 500 mL with 0.005 M HCl. Bubble solution with nitrogen gas.

7.3 Working standards: Prepare a 100 mg/L ferrous iron standard from the 1,000 mg/L stock by the diluting the stock 1:10. Use 0.0005 M HCl for all dilutions. Prepare 100 mL of each of the working standards (0.5, 1.0, 5.0, 10 and 15 mg/L standards) from the 100 mg/L secondary standard.

7.4 For soil samples extract samples with 0.5 M HCl (42 mL of concentrated HCl per liter).

8.0 Quality Control

8.1 QC Samples

Method blank (MB), matrix spike (MS), matrix spike duplicate (MSD), laboratory control samples (LCS), and laboratory control sample duplicates (LCSD) should be analyzed on a daily basis or each analytical batch (20 samples or less).

8.2 QC Limits

8.2.1 Before any samples are analyzed, it should be demonstrated with a method blank, that the system is reasonably free of contamination that would interfere with the determination of any analytes of interest.

8.2.2 The initial calibration curve should have a correlation coefficient, r, of at least 0.995 with a minimum of three concentration points plus a blank. The control limit for initial calibration verification (ICV) is 90% - 110%.

8.2.3 Perform the daily or continuing calibration verification (CCV) by measuring the midpoint calibration before sample analysis of every 10 samples. The control limit for the CCV is 95%-105%.

8.2.4 The recoveries of the analytes in MS, MSD, and LCS should be within the following control limits: the control limit of lab control samples is 80%-120%; and the control limit for the matrix spikes is 75%-125%.

8.2.5 All calibration and method blanks should have concentrations of analytes less than the practical quantitation limit (PQL).

8.3 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL).

Analyte	MDL (µg/L)	Date	PQL (µg/L)	Source
Ferrous iron	12.0	7/10/01	20.0	UWRL

9.0 Corrective Action

9.1 If the method blank (or instrument blank) indicates a result higher than the PQL, the containers, reagents, and analytical system should be carefully examined and cleaned until the background disappears before samples can be analyzed.

9.2 If the CCV sample is outside of the control limit, reanalyze the CCV. If the CCV is still outside of the control limit, perform a new initial calibration and reanalyze all samples back to the last acceptable CCV.

9.3 If recoveries of the LCS are outside the acceptable range, reanalyze the LCS. If the recovery is still outside the acceptable range, carefully examine the analysis process and correct any problems that may have occurred. Reanalyze the associated sample batch.

9.4 If there is not enough sample for a MSD or even for a MS, the LCS/LCSD may be used for the QC report.

9.5 If one of the recoveries of the MS and MSD is outside of the control limit check the average of the MS and MSD. If the average is also out of control, perform one more matrix spike.

9.6 If recoveries of both of the MS and MSD are outside the required range, check the recoveries of the LCS. If the LCS recoveries are reasonable, matrix interference is suspected and should be controlled as discussed in Section 3; otherwise reanalyze the associated sample batch.

10.0 Record Keeping and Storage

All raw data, such as chain of custody, sample preparation record, analysis logbook and the analytical data, etc., will be kept in file for a minimum of 5 years from the date the report is sent to the client.

11.0 References

Lovley, D.R. and E.P. Phillips. 1986. Organic matter mineralization with the reduction of ferric iron in anaerobic sediments. *Appl. and Environ. Micro.* **51**:683-68.

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Appendix I. SOP I-5 Determination of Arsenic(III) Using an Anion Exchange Resin and Atomic Absorption Spectroscopy, Furnace Technique
Method: EPA 7060A (GFAA)

File Name I-05 AA AsIII SOP Ver. 1.1
Version No 1.1
Revision Date 7/2001

Prepared/ Revised by:

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Approval:

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QA Officer

R. Ryan Dupont
Division Head, Environmental Engineering

I-5 Determination of Arsenic(III) Using an Anion Exchange Resin and Atomic Absorption Spectroscopy, Furnace Technique Method: EPA 7060A (GFAA)

1.0 Scope and Application

1.1 This method covers the analysis of As(III).

1.2 The matrices applicable to this method are drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, and leachates.

2. Summary of Method

2.1 Arsenic(III) at pH 4.0 is fully protonated, whereas As(V) is fully deprotonated. A liquid sample, after acidification with sulfuric acid to pH 4, is passed through a column packed with Dowex 1 x 8 anion exchange resin (50-100 mesh) (Bio-Rad). The charged As(V) is sorbed to the resin and the As(III) passes through the column and is collected, acidified to pH < 2 with nitric acid and is analyzed for As by graphite furnace atomic absorption spectroscopy (GFAA). The GFAA analysis for As is given in Method I-4A.

3.0 Interferences

3.1 See Section 3.0 Method I-4A.

3.2 Arsenic(III) is the reduced form of arsenic. Samples must be protected from exposure to oxygen or other oxidizing agents (e.g., HNO_3) during collection, storage and analysis. Samples should be analyzed immediately after collection. Sample preparation may need to be carried out in a glove box with a nitrogen-hydrogen atmosphere to completely exclude oxygen.

4.0 Apparatus

4.1 Perkin Elmer Aanalyst 800 Atomic Absorption Spectrometer.

4.2 Instrument parameters Arsenic:

4.2.1 Arsenic electrodeless discharge lamp.
4.2.2 Wavelength: 193.7 nm.
4.2.3 Slit 0.7 Low.
4.2.4 Background correction required.
4.2.5 Pretreatment temperature: 1,200 °C.
4.2.6 Atomization temperature: 2,000°C.
4.2.7 Poly-prep 1.5 x 12 cm polypropylene chromatography columns (Bio-Rad).

5.0 Reagents

5.1 Dowex 1 x 8 anion exchange resin (20-50 mesh) (Bio-Rad).
5.2 Nitric acid.
5.3 Sulfuric acid.
5.4 Acetic acid.
5.5 See Section 5.0 Method I-4A.

6.0 Sample Collection, Preservation and storage

6.1 See Section 6.0 Method I-4.
6.2 Arsenic(III) is the reduced form of arsenic. Samples must be protected from exposure from oxygen or other oxidizing agents during collection, storage and analysis. Samples should be analyzed immediately after collection. Sample preparation may need to be carried out in a glove box with a nitrogen-hydrogen atmosphere to completely exclude oxygen.

7.0 Procedure

7.1 Resin Preparation: Dowex 1 x 8 anion exchange resin (20-50 mesh) (Bio-Rad) is converted from its chloride to its acetate form by equilibrating the resin in batch reactors with 1 M sodium hydroxide followed by rinsing with DI water, followed by four equilibration steps with 1 M acetic acid, followed by rinsing with DI water.
7.1.1 200 g of resin is weighed into a 1-L beaker containing 200 mL 1 M NaOH. The slurry is stirred for 1 hour using a magnetic stir bar and stir plate. The NaOH solution is removed from the resin by filtration using a Buchner

funnel with Whatman No. 1 filter paper. This process is repeated three more times.

7.1.2 The resin is then rinsed with two 200 mL portions of DI water.

7.1.3 Transfer the resin to a beaker, add 200 mL of 1 N acetic acid and stir for 5 minutes. Filter as described above. Repeat process three more times.

7.1.4 Filter and rinse the resin with 200 mL DI water.

7.1.5 Column Preparation: Columns are packed with 8 g of prepared resin. When packed replace end caps and store under refrigeration.

7.2 The water sample (10 mL) is first filtered through a 0.45 µm filter into a snap cap vial containing 0.2 mL of 1 % sulfuric acid. This concentration of sulfuric acid is sufficient to lower the pH of the water to pH 3.5. For water samples from a source other than Hill Air Force Base, the concentration of acid will have to be determined by trial and error. A 5mL sample is passed through the column and the effluent is discarded. An additional 5 mL are passed through the column and are collected into a snap cap vial. Acidify this sample to pH < 2 with nitric acid. The neutral As(III) species pass through the column and the anionic As(V) remain on the column. Store sample for analysis of As by GFAA (refer to Section 7.0 Method I-04 for GFAA analysis procedures).

8.0 Quality Control

8.1 See Section 8.0 Method I-4.

8.2 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL (µg/L)	Date	PQL(µg/L)	Source
Arsenic(GFAA)	0.80	7/10/01	1.33	UWRL

9.0 Corrective Action

9.1 See Section 9.0 Method I-4.

10.0 Record Keeping and Storage

10.1 See Section 10 Method I-4.

11.0 References

Chiu, V, Q. and J. G. Hering. 2000. Arsenic adsorption and oxidation at manganite surfaces. 1. Method of adsorbed and dissolved arsenic species. *Environ. Sci. Technol* **34**:2029-2034.

Edwards, M., S. Patel, L. McNeill, H-W Chen, M. Frey, A. D. Eaton, R. C. Antweiler, and H. E. Taylor. 1998. Considerations in As analysis and speciation. *J AWWA* **90**:103-113.

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Appendix J. SOP I-4A Determination of Arsenic by Atomic Absorption Spectroscopy, Furnace Technique Method: EPA 7060A

File Name I-04A AA As SOP Ver. 1.1
Version No 1.1
Revision Date 7/2001

Prepared/ Revised by:

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Approval:

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R. Ryan Dupont
Division Head, Environmental Engineering

I-4A Determination of Arsenic by Atomic Absorption Spectroscopy, Furnace Technique Method: EPA 7060A

1.0 Scope and Application

1.1 See Section 1.0 Method I-4.

2.0 Summary of Method

2.1 See Section 2.0 Method I-4.

2.2 Prior to analysis sample may need to be acid digested to convert organic forms of As to inorganic forms.

3.0 Interferences

3.1 See Section 3.0 Method I-4.

3.2 Elemental As and many of its compounds are volatile; therefore samples may be subject to lose of As during sample preparation. Spiked sample and relevant standard reference material should be processed to determine if the chosen dissolution method is appropriate.

3.3 Palladium plus magnesium nitrate is used as the matrix modifier. Observe the shape and height of the analytical signal using a select sample from each type of matrix encountered. The curve should be Gaussian in shape, with no fronting or tailing. Change the ash temperature in 1,000 °C intervals and observe the effect on peak shape and area, and background peak area. Once the best conditions are selected, change the atomization temperature in 1,000 °C intervals, again optimizing the output.

3.4 Zeeman effect background correction must be used.

4.0 Apparatus

4.1 Perkin Elmer Aanalyst 800 Atomic Absorption Spectrometer.

4.2 Instrument parameters Arsenic:

4.2.1 Arsenic electrodeless discharge lamp.
4.2.2 Wavelength: 193.7 nm.
4.2.3 Slit: 0.7 Low.
4.2.4 Background correction required.
4.2.5 Pretreatment temperature: 1,200°C.
4.2.6 Atomization temperature: 2,000 °C.

5.0 Reagents

5.1 See Section 5.0 Method I-4.
5.2 To prepare the matrix modifier, 0.3 mL of 1% Mg nitrate are added to 5 mL of 1,000 mg/L Pd. The Pd solution is a certified standard available from Perkin Elmer or other vendors.

6.0 Sample Collection, Preservation and storage

6.1 See Section 6.0 Method I-4.

7.0 Procedure

7.1 See Section 7.0 Method I-4.

8. Quality Control

8.1 See Section 8.0 Method I-4.
8.2 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL (µg/L)	Date	PQL (µg/L)	Source
Arsenic(GFAA)	0.80	7/10/01	1.33	UWRL

9.0 Corrective Action

9.1 See Section 9.0 Method I-4.

10.0 Record Keeping and Storage

10.1 See Section 10.0 Method I-4.

11.0 References

EPA Method 7060A, Revision 1, 1994.

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Appendix K. SOP I-4B Determination of Iron and Manganese by Atomic Absorption Spectroscopy, Direct Aspiration Method: EPA 7380 and 7460

File Name I-04B AA Fe/Mn SOP Ver. 1.1
Version No 1.1
Revision Date 7/2001

Prepared/ Revised by:

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I-4B Determination of Iron and Manganese Atomic Absorption Spectroscopy, Direct Aspiration Method: EPA 7380 and 7460

1.0 Scope and Application

1.1 See Section 1. Method I-4.

2.0 Summary of Method

2.1 See Section 2.0 Method I-4.

3.0 Interferences

3.1 See Section 3.0 Method I-4.

3.2 Iron is a universal contaminant and great care should be taken to avoid contamination. Background correct should be used for both Fe and Mn analysis.

4.0 Apparatus

4.1 Perkin Elmer Aanalyst 800 Atomic Absorption Spectrometer.

4.2 Instrument parameters Iron:

- 4.2.1 Iron hallow cathode lamp.
- 4.2.2 Wavelength: 248.3 nm.
- 4.2.3 Fuel: acetylene.
- 4.2.4 Oxidant: Air.
- 4.2.5 Type of Flame: oxidizing (Fuel, lean).
- 4.2.6 Background correction required.

4.3 Instrument parameters Manganese:

- 4.3.1 Manganese hallow cathode lamp.
- 4.3.2 Wavelength: 279.5 nm.
- 4.3.3 Fuel: acetylene.
- 4.3.4 Oxidant: Air.
- 4.3.5 Type of Flame: oxidizing (Fuel, lean).
- 4.3.6 Background correction required

5.0 Reagents

5.1 See Section 5.0 Method I-4.

6.0 Sample Collection, Preservation and Storage

6.1 See Section 6.0 Method I-4.

7.0 Procedure

7.1 See Section 7.0 Method I-4.

8.0 Quality Control

8.1 See Section 8.0 Method I-4.

8.2 Method Detection Limit (MDL), and Practical Quantitation Limit (PQL)

Analyte	MDL (µg/L)	Date	PQL (µg/L)	Source
Iron (FAA)	60	7/10/01	100	UWRL
Manganese (FAA)	30	7/10/01	50	UWRL

9.0 Corrective Action

9.1 See Section 9.0 Method I-4.

10. Record Keeping and Storage

10.1 See Section 10.0 Method I-4.

11.0 References

EPA Method 7380, Revision 0, 1986; Method .7460, Revision 0, 1986.

Appendix L
BR Dilution Study

Treat	Nutrient	Biotic	Event	Rep	E1, t=0				E2, t=3				E2-3, t=7				E2-4, t=14				E2-5, t=21			
					TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)
Undiluted	1	1		1	11.173	1.343	0.01	0.11	9.30	1.227	0.01	0.11	10.28	1.237	0.01	0.12					0.0003	5.6442	0.0055	0.3292
Undiluted	1	1		2	10.943	1.287	0.01	0.10	9.46	1.311	0.01	0.12	10.383	1.3018	0.0081	0.1245	0.0	9.5706	0.0	0.239	0.0003	5.4893	0.0054	0.3316
Undiluted	1	1		3	10.92	1.279	0.01	0.10	9.57	1.306	0.01	0.12	10.269	1.2831	0.0079	0.1204	0.0	8.9847	0.0	0.217	0.0003	5.2583	0.0053	0.3235
					11.01	1.30	0.01	0.10	9.44	1.28	0.01	0.12	10.31	1.27	0.01	0.12	0.0	9.28	0.0	0.23	0.00	5.46	0.01	0.33
					0.14	0.03	0.00	0.00	0.14	0.05	0.00	0.01	0.06	0.03	0.00	0.00	0.0	0.41	0.0	0.02	0.00	0.19	0.00	0.00
					1.3	2.7	3.7	3.7	1.4	3.6	19.9	7.9	0.61	2.6	2.3	1.7	#DIV/0!	4.47	#DIV/0!	7.07	3.77	3.55	1.79	1.28
					0.2	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.07	0.0	0.0	0.0	#NUM!	0.57	#NUM!	0.02	0.00	0.22	0.00	0.00
1:4	1	1		1	8.465	0.198	0.0	0.012					7.7949	0.1825	0.002	0.0095	5.4836	1.9938	0.0	0.0	0.0002	5.9542	0.0022	0.013
1:4	1	1		2	8.3035	0.196	0.0	0.011					7.9332	0.1867	0.0017	0.0096	6.0079	1.7664	0.0	0.0	0.0002	5.9281	0.0019	0.0123
1:4	1	1		3	8.829	0.207	0.0	0.011					8.1068	0.1946	0.0017	0.0096	5.2371	2.5149	0.0	0.0	0.0002	5.9784	0.002	0.011
					8.53	0.20	0.0	0.01					7.94	0.19	0.00	0.01	5.58	2.09	0.0	0.0	0.00	5.95	0.00	0.01
					0.27	0.01	0.0	0.00					0.16	0.01	0.00	0.00	0.39	0.38	0.0	0.0	0.00	0.03	0.00	0.00
					3.2	2.88	#DIV/0!	3.45					1.97	3.27	8.88	0.89	7.06	18.34	#DIV/0!	#####	9.32	0.42	6.60	8.22
					0.30	0.01	#NUM!	0.00					0.18	0.01	0.00	0.00	0.45	0.43	#NUM!	#NUM!	0.00	0.03	0.00	0.00
1:10	1	1		1	8.29	0.053	0.0	0.003					7.59	0.046	0.0	0.0	4.1302	2.0996	0.0	0.0	0.0005	4.9517	0.0007	0.0016
1:10	1	1		2	8.15	0.047	0.0	0.000					7.1115	0.0403	0.0	0.0	3.7268	2.5022	0.0	0.0	0.0004	5.0053	0.0007	0.0021
1:10	1	1		3	8.25	0.047	0.0	0.002					7.4252	0.0378	0.0	0.0	5.6449	1.3129	0.0	0.0	0.0004	5.3162	0.0008	0.0016
					8.23	0.05	0.0	0.00					7.38	0.04	0.0	0.0	4.50	1.97	0.0	0.0	0.00	5.09	0.00	0.00
					0.07	0.00	0.0	0.00					0.24	0.00	0.0	0.0	1.01	0.60	0.0	0.0	0.00	0.20	0.00	0.00
					0.9	7.3	#DIV/0!	87.5					3.32	10.4	#DIV/0!	#DIV/0!	22.47	30.68	#DIV/0!	#####	16.01	3.87	6.15	15.85
					0.1	0.0	#NUM!	0.0					0.28	0.0	#NUM!	#NUM!	1.14	0.68	#NUM!	#NUM!	0.00	0.22	0.00	0.00
1:100	1	1		1	7.90	0.003	0.0	0.0					7.4434	0.003	0.0	0.0	7.2155	0.119	0.0	0.0	5.841	0.6731	0.0005	0.0003
1:100	1	1		2	8.3505	0.003	0.0	0.0					7.3959	0.0032	0.0	0.0	6.5758	0.3118	0.0	0.0	5.4974	0.8684	0.0011	0.0003
1:100	1	1		3	8.418	0.003	0.0	0.0					7.8135	0.0038	0.0	0.0	7.0929	0.0	0.0	0.0	0.1707	4.8706	0.0012	0.0007
					8.22	0.00	0.0	0.0					7.55	0.00	0.0	0.0	6.96	0.14	0.0	0.0	3.84	2.14	0.00	0.00
					0.28	0.00	0.0	0.0					0.23	0.00	0.0	0.0	0.34	0.16	0.0	0.0	3.18	2.37	0.00	0.00
					3.4	7.47	#DIV/0!	#####					3.03	11.38	#DIV/0!	#DIV/0!	4.88	109.59	#DIV/0!	#####	82.87	110.84	43.33	42.34
					0.32	0.00	#NUM!	#NUM!					0.26	0.00	#NUM!	#NUM!	0.38	0.18	#NUM!	#NUM!	3.60	2.68	0.00	0.00
1:1000	1	1		1	8.213	0.0	0.0	0.0					7.5683	0.0	0.0	0.0	6.9278	0.0	0.0	0.0	5.8578	0.0016	0.0002	0.0003
1:1000	1	1		2	8.2065	0.0	0.0	0.0					7.5518	0.0	0.0	0.0	7.1669	0.0	0.0	0.0	0.7858	4.2534	0.0004	0.0003
1:1000	1	1		3	8.042	0.0	0.0	0.0					7.4808	0.0	0.0	0.0	7.1741	0.0	0.0	0.0	4.276	1.7203	0.0013	0.0003
					8.15	0.0	0.0	0.0					7.53	0.0	0.0	0.0	7.09	0.0	0.0	0.0	3.64	1.99	0.00	0.00
					0.10	0.0	0.0	0.0					0.05	0.0	0.0	0.0	0.14	0.0	0.0	0.0	2.60	2.14	0.00	0.00
					1.2	#####	#DIV/0!	#####					0.62	#DIV/0!	#DIV/0!	#DIV/0!	1.98	#DIV/0!	#DIV/0!	#####	71.30	107.39	96.71	12.48
					0.11	#NUM!	#NUM!	#NUM!					0.05	#NUM!	#NUM!	#NUM!	0.16	#NUM!	#NUM!	#NUM!	2.94	2.42	0.00	0.00

Appendix M

Treat	Nutrient	Biotic	Event	Rep	E1, t=0 TCE (mg/l	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	E2 TCE (mg/l	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	Treat	Nutrient	Biotic	Event	Rep	E3 TCE (mg/	cis-DCE (mg/	trans-DCE (mg/L)	VC (mg/L)	E4 TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	E5 TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)
Gran X	1	1		1	2.42	0.001	0.0	0.0002	2.90	0.004	0.001	0.0	Gran X	1	1		1	2.75	0.23	0.00244	0.0016	0.0	1.4827	0.0	0.0	0.0	0.66246	0.0	0.0422
Gran X	1	1		2	2.49	0.001	0.0	0.0002	2.95	0.004	0.001	0.0	Gran X	1	1		2	2.65	0.38	0.00241	0.0021	0.0	1.4444	0.0	0.0				
Gran X	1	1		3	2.49	0.001	0.0	0.0003	2.74	0.004	0.001	0.0	Gran X	1	1		3	3.02	0.08	0.00233	0.0015	0.0	1.4787	0.0	0.0				
Gran X	1	0		1	2.53	0.000	0.0	0.0	2.84	0.0	0.0	0.0	Gran X	1	0		1	2.54	0	0	0	2.437	0	0.0	0.0	1.965		0.0	0.0
Gran X	1	0		2	2.39	0.000	0.0	0.0	2.84	0.0	0.0	0.0	Gran X	1	0		2	2.51	0	0	0	2.473	0	0.0	0.0	1.974		0.0	0.0
Gran X	1	0		3	2.38	0.000	0.0	0.0	2.81	0.0	0.0	0.0	Gran X	1	0		3	2.49	0	0	0	2.427	0	0.0	0.0	1.996		0.0	0.0
					2.47	0.0	0.0	0.0	2.86	0.0	0.0	0.0						2.80	0.23	0.00	0.00	0.00	1.47	0.0	0.0	0.0	0.66	0.0	0.04
					0.04	0.0	0.0	0.0	0.11	0.0	0.0	0.0						0.19	0.15	0.00	0.00	0.00	0.02	0.0	0.0	#####	#DIV/0!	#DIV/0!	#DIV/0!
					1.7	11.5	#DIV/0!	8.7	3.7	1.3	2.7	16.2						6.8	63.4	2.4	18.7	#####	1.4	#DIV/0!	######	#####	#DIV/0!	#DIV/0!	#DIV/0!
					0.0	0.0	#NUM!	0.0	0.1	0.0	0.0	0.0						0.2	0.2	0.0	0.0	#####	0.0	#NUM!	#NUM!	#####	#DIV/0!	#DIV/0!	#DIV/0!
X	1	1		1	2.93	0.0	0.0	0.0	3.36	0.0	0.0	0.0	X	1	1		1	2.88	0	0.0	0.0	3.082	0.0	0.0	0.0	2.424		0.0	0.0
X	1	1		2	2.98	0.0	0.0	0.0	3.29	0.0	0.0	0.0	X	1	1		2	3.06	0	0.00017	0.0	3.024	0.0	0.0	0.0	2.606		0.0	0.0
X	1	1		3	2.95	0.0	0.0	0.0	3.27	0.0	0.0	0.0	X	1	1		3	3.01	0	0.0	0.0	2.942	0.0	0.0	0.0	2.451		0.0	0.0
X	1	0		1	2.85	0.0	0.0	0.0	3.28	0.0	0.0	0.0	X	1	0		1	3	0	0.0	0.0	2.899	0.0	0.0	0.0	2.526		0.0	0.0
X	1	0		2	3	0.0	0.0	0.0	3.35	0.0	0.0	0.0	X	1	0		2	3	0	0.0	0.0	2.79	0.0	0.0	0.0	2.191		0.0	0.0
X	1	0		3	3.05	0.0	0.0	0.0	3.29	0.0	0.0	0.0	X	1	0		3	3	0	0.0	0.0	3.06	0.0	0.0	0.0	2.186		0.0	0.0
					2.95	0.0	0.0	0.0	3.30	0.0	0.0	0.0						2.98	0.0	0.0	0.0	3.02	0.0	0.0	0.0	2.49	#DIV/0!	0.0	0.0
					0.03	0.0	0.0	0.0	0.05	0.0	0.0	0.0						0.09	0.0	0.0	0.0	0.07	0.0	0.0	0.0	0.10	#DIV/0!	0.0	0.0
					0.9	2.8	173.2	#DIV/0!	1.4	13.0	158.85	######						3.1	10.9	173.2	#DIV/0!	2.3	#DIV/0!	#DIV/0!	######	4.0	#DIV/0!	#DIV/0!	#DIV/0!
					0.0	0.0	0.0	#NUM!	0.1	0.0	0.00	#NUM!						0.1	0.0	0.0	#NUM!	0.1	#NUM!	#NUM!	#NUM!	0.1	#DIV/0!	#NUM!	#NUM!

Treat	Nutrient	Biotic	Event	Rep	E2-1 t=0	E2-2				E2-3				E2-4				E2-5			
					TCE (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)	TCE (mg/L)	cis-DCE (mg/L)	trans-DCE (mg/L)	VC (mg/L)
Control	1	1		1	6.445	3.80	0.002	0.000	0.000	3.00	0.000	0.000	0.000	1.4253	0	0	0	1.00459	0	0	0
	1	1		2	6.467	3.75	0.002	0.000	0.000	3.01	0.000	0.000	0.000	1.4758	0	0	0	0.93826	0	0	0
	1	1		3	6.541	3.94	0.002	0.000	0.000	3.03	0.000	0.000	0.000	1.3823	0	0	0	1.06419	0	0	0
	1	0		1	6.782	4.10	0.000	0.000	0.000	2.88	0.000	0.000	0.000	1.5438	0	0	0	1.01965	0	0	0
	1	0		2	7.026	4.04	0.000	0.000	0.000	2.56	0.000	0.000	0.000	1.3977	0	0	0	1.0135	0	0	0
	1	0		3	6.472	4.22	0.000	0.000	0.000	3.02	0.000	0.000	0.000	1.5976	0	0	0	1.02358	0	0	0
	1	1	0	1		3.80	0.000	0.000	0.000	2.92	0.000	0.000	0.000	1.4366	0	0	0	1.11925	0	0	0
	1	1	0	2	6.434	3.61	0.000	0.000	0.000	3.09	0.000	0.000	0.000	1.4879	0	0	0	1.10519	0	0	0
	1	1	0	3	6.653	4.02	0.000	0.000	0.000	3.14	0.000	0.000	0.000	1.6095	0	0	0	1.05938	0	0	0
	1	0	0	1	6.734	3.78	0.000	0.000	0.000	3.05	0.000	0.000	0.000	1.6839	0	0	0	0.625	0	0	0
	1	0	0	2	6.559	4.04	0.000	0.000	0.000	2.76	0.000	0.000	0.000	1.4554	0	0	0	0.59229	0	0	0
	1	0	0	3	6.716	4.06	0.000	0.000	0.000	2.92	0.000	0.000	0.000	1.6441	0	0	0	0.98527	0	0	0
					6.62	3.97	0.00	0.00	0.00	2.92	0.00	0.00	0.00	1.47	0.00	0.00	0.00	1.01	0.00	0.00	0.00
					0.18	0.18	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.04	0.00	0.00	0.00
					2.8	4.51	109.72	#DIV/0!	#DIV/0!	6.31	#DIV/0!	#DIV/0!	#DIV/0!	5.82	#DIV/0!	#DIV/0!	#DIV/0!	4.06	#DIV/0!	#DIV/0!	#DIV/0!
					0.1	0.14	0.00	#NUM!	#NUM!	0.15	#NUM!	#NUM!	#NUM!	0.07	#NUM!	#NUM!	#NUM!	0.03	#NUM!	#NUM!	#NUM!
Whey	1	1		1	7.177	6.16	0.007	0.000	0.000	3.717	0.010	0	0	2.6347	0.02418	0.00104	0	2.15809	0.04245	0.00348	0.00133
	1	1		2	6.474	6.17	0.008	0.000	0.000	3.23	0.01332	0	0	2.6533	0.02339	0.00133	0	2.02942	0.11041	0.00578	0.00113
	1	1		3	7.497	6.20	0.008	0.000	0.000	3.45	0.0147	0	0	2.6133	0.0219	0.00108	0	1.97119	0.0527	0.00243	0.00106
	1	0		1	7.096	5.90	0.007	0.000	0.000	3.424	0.00123	0	0	2.7739	0.02227	0.00151	0	2.14744	0.05691	0.00198	0.00083
	1	0		2	7.147	6.30	0.007	0.000	0.000	3.395	0.01086	0.00077	0	3.0901	0.01473	0.00122	0	2.14515	0.06274	0.00374	0.0013
	1	0		3	6.826	6.15	0.007	0.000	0.000	3.697	0.00904	0	0	3.2427	0.01591	0	0	2.19072	0.04878	0.00324	0.00107
	1	1	0	1	6.787	6.29	0.004	0.000	0.000	4.109	0.00637	0	0	3.7245	0.002	0	0	2.34774	0.00276	0	0
	1	1	0	2	6.709	5.98	0.004	0.000	0.000	3.964	0.00245	0	0	3.923	0.00237	0	0	2.17722	0.00238	0.00035	0
	1	1	0	3	6.778	6.14	0.003	0.000	0.000	3.966	0.00249	0	0	4.0786	0.00227	0	0	2.33763	0.002	0	0
	1	0	0	1	6.916	6.23	0.004	0.000	0.000	4.109	0.00258	0	0	3.7837	0.00213	0	0	2.23488	0.01755	0	0
	1	0	0	2	7.155	6.39	0.003	0.000	0.000	3.149	0.00199	0	0	4.024	0.00228	0	0	2.39377	0.00201	0	0
	1	0	0	3	7.443	6.02	0.004	0.000	0.000	4.057	0.00249	0	0	3.9844	0.00231	0	0	2.39402	0.00207	0	0
					7.00	6.15	0.01	0.00	0.00	3.49	0.01	0.00	0.00	2.83	0.02	0.00	0.00	2.11	0.06	0.00	0.00
					0.30	0.13	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.09	0.02	0.00	0.00
					4.4	2.14	6.63	#DIV/0!	#DIV/0!	5.40	48.14	244.95	#DIV/0!	9.43	19.77	51.74	#DIV/0!	4.09	39.38	38.46	16.31
					0.2	0.11	0.00	#NUM!	#NUM!	0.15	0.00	0.00	#NUM!	0.21	0.00	0.00	#NUM!	0.07	0.02	0.00	0.00
Coconu	1	1		1	7.468	4.638	0.0053	0.01329	0.000	2.897	0.00401	0.00882	0	1.3705	0	0	0	1.22634	0	0	0
	1	1		2	7.151	4.72	0.004	0.006	0.000	2.964	0.00441	0.00373	0	1.2182	0	0	0	1.31695	0	0	0
	1	1		3	7.019	4.71	0.004	0.002	0.000	2.926	0.004	0.00838	0	1.4871	0	0	0	1.11102	0	0.02061	0
	1	0		1	6.924	4.67	0.004	0.000	0.000	2.944	0.00387	0.01499	0	1.5146	0	0	0	1.16803	0	0	0
	1	0		2	7.129	4.52	0.004	0.000	0.000	2.645	0.00352	0.01008	0	1.3743	0	0	0	1.37826	0	0	0
	1	0		3	7.643	4.54	0.004	0.000	0.000	2.914	0.00364	0.00494	0	1.3065	0	0	0	1.22265	0	0	0
	1	1	0	1	7.317	4.59	0.003	0.000	0.000	2.964	0.0026	0	0	1.6193	0	0	0	1.23348	0	0	0
	1	1	0	2	7.107	4.52	0.003	0.000	0.000	2.955	0.00264	0	0	1.3243	0	0	0	1.38066	0	0	0
	1	1	0	3	7.113	4.50	0.003	0.000	0.000	2.833	0.00253	0	0	1.4743	0	0	0	1.39761	0	0	0
	1	0	0	1	7.288	4.67	0.003	0.000	0.000	2.856	0.00258	0	0	1.4337	0	0	0	1.09528	0	0	0
	1	0	0	2	7.031	4.47	0.003	0.000	0.000	2.958	0.00269	0	0	1.2477	0	0	0	1.16408	0	0	0
	1	0	0	3	7.26	4.66	0.003	0.000	0.000	3.025	0.00269	0	0	1.5528	0	0	0	1.27427	0	0	0
					7.20	4.63	0.00	0.00	0.00	2.88	0.00	0.01	0.00	1.38	0.00	0.00	0.00	1.24	0.00	0.00	0.00
					0.20	0.09	0.00	0.01	0.00	0.12	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.10	0.00	0.01	0.00
					2.8	1.86	15.21	151.16	#DIV/0!	4.11	8.05	47.16	#DIV/0!	8.03	#DIV/0!	#DIV/0!	#DIV/0!	7.86	#DIV/0!	244.95	#DIV/0!

Appendix O
EC Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-1	E2-2	E2-3	E2-4	E2-5	E2-6
Microcosm No.					As (µg/L)	As (µg/L)	As (µg/L)	As (µg/L)	As (µg/L)	As (µg/L)
Control	1	1	2-#	1	3.33	2.44				
1	1	1	2-#	2	3.28	1.74	5.09			
1	1	1	2-#	3	3.70	1.74	3.65			
1	0	1	2-#	1	3.11	1.70	2.99			
1	0	1	2-#	2	3.14	1.42	4.60			
1	0	1	2-#	3	2.73	3.16	3.55			
1	1	0	2-#	1	2.21	3.19	5.12			
1	1	0	2-#	2	2.18		4.93			
1	1	0	2-#	3	3.21	3.34	3.64			
1	0	0	2-#	1	2.73	1.77	3.22			
1	0	0	2-#	2	2.34	1.99	2.40			
1	0	0	2-#	3	2.22	1.43	3.76			
Whey	1	1	2-#	1	16.3	24.78	34.93			
4	1	1	2-#	2	15.7	20.34	93.59			
4	1	1	2-#	3	10.8	21.03	43.82			
4	0	1	2-#	1	10.0	20.61	41.44			
4	0	1	2-#	2	14.0	20.45	52.70			
4	0	1	2-#	3	13.2	21.99	63.96			
4	1	0	2-#	1	9.2	21.87	26.83			
4	1	0	2-#	2	8.8	20.65	27.18			
4	1	0	2-#	3	10.7	21.46	25.20			
4	0	0	2-#	1	7.3	21.23	19.95			
4	0	0	2-#	2	6.9	20.06	34.95			
4	0	0	2-#	3	8.9	42.10	24.81			
Coconut Oil	1	1	2-#	1	2.45	22.69	23.33			
7	1	1	2-#	2	2.76	30.55	19.39			
7	1	1	2-#	3	3.04	23.84	30.02			
7	0	1	2-#	1	2.95	25.89	32.81			
7	0	1	2-#	2	2.50	24.94	23.70			
7	0	1	2-#	3	2.66	25.90	26.06			
7	1	0	2-#	1	3.18	3.02	4.39			
7	1	0	2-#	2	5.14	8.79	9.27			
7	1	0	2-#	3	2.47	1.96	2.65			
7	0	0	2-#	1	2.23	2.33	2.53			
7	0	0	2-#	2	2.37	3.07	2.52			
7	0	0	2-#	3	3.21	2.69	4.77			
Emulsified Oi	1	1	2-#	1	2.62					55.99
X	1	1	2-#	2	3.61					63.46
X	1	1	2-#	3	4.14					69.16
X	1	0	2-#	1	4.21					12.88
X	1	0	2-#	2	3.37					12.65
X	1	0	2-#	3	3.95					2.86

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-4 CH4 (Vol%)	E2-4 Ethane (vol%)	E2-4 Ethene (vol%)	E2-4 CO2 (Vol%)	E2-5 CH4 (Vol%)	E2-5 Ethane (vol%)	E2-5 Ethene (vol%)	E2-5 CO2 (Vol%)	E2-6 CH4 (Vol%)	E2-6 Ethane (vol%)	E2-6 Ethene (vol%)	E2-6 CO2 (Vol%)
A	1	1	2-#	1												
A	1	1	2-#	2												
A	1	1	2-#	3												
A	1	0	2-#	1												
A	1	0	2-#	2												
A	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
B	1	1	2-#	1	0.002037	0	0	1.33929								
B	1	1	2-#	2	0.002928	0.002304	0	1.17208								
B	1	1	2-#	3	0.001903	0.0023356	0	0.71878								
B	1	0	2-#	1	0.002006	0.0023106	0	0								
B	1	0	2-#	2	0.002009	0.0023044	0	0.37125								
B	1	0	2-#	3	0.002027	0.0023078	0	0.37206								
					0.00	0.00	0.00	1.08	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					0.00	0.00	0.00	0.32	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					24.34	86.61	#DIV/0!	29.82	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					0.00	0.00	#NUM!	0.36	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
C	1	1	2-#	1					18.89809	0	0.0007033		0.007551	0.002348	0	33.746
C	1	1	2-#	2					15.60519	0	0.0006089	11.58148	0.0082684	0.002277	0	34.574
C	1	1	2-#	3					16.34303	0	0.000578	12.1517	0.008239	0.002357	0	33.575
C	1	0	2-#	1					0.439867	0	0.0004221	11.52294	0.0020198	0.002298	0	26.628
C	1	0	2-#	2					0.632747	0	0.0003688	10.13173	0.0020301	0.002328	0	27.933
C	1	0	2-#	3					1.18057	0	0.000508	10.91144	0.0020044	0.002363	0	27.617
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	16.95	0.00	0.00	11.87	0.01	0.00	0.00	33.97
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.73	0.00	0.00	0.40	0.00	0.00	0.00	0.53
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	10.20	#DIV/0!	10.37	3.40	5.06	1.88	#DIV/0!	1.57
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.96	#NUM!	0.00	0.56	0.00	0.00	#NUM!	0.60
D	1	1	2-#	1					50.02723	0	0.0003914	10.63377				
D	1	1	2-#	2												
D	1	1	2-#	3												
D	1	0	2-#	1					0.222662	0	0.0004775	2.47927				
D	1	0	2-#	2					0.215083	0	0.0004797	2.56792				

Appendix P

TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-4 CH4 (Vol%)	E2-4 Ethane (vol%)	E2-4 Ethene (vol%)	E2-4 CO2 (Vol%)	E2-5 CH4 (Vol%)	E2-5 Ethane (vol%)	E2-5 Ethene (vol%)	E2-5 CO2 (Vol%)	E2-6 CH4 (Vol%)	E2-6 Ethane (vol%)	E2-6 Ethene (vol%)	E2-6 CO2 (Vol%)
D	1	0	2-#	3					0.23167	0	0.0003954	3.63351				
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	50.03	0.00	0.00	10.63	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	######
E	1	1	2-#	1									19.23586	0.002942	0	38.622
E	1	1	2-#	2									19.10526	0.002966	0	37.908
E	1	1	2-#	3									21.2141	0.002875	0	36.183
E	1	0	2-#	1									0.0034086	0.002562	0	32.525
E	1	0	2-#	2									0.0034509	0.002557	0	31.33
E	1	0	2-#	3									0.0030672	0.002533	0	31.742
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	19.85	0.00	0.00	37.57
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.18	0.00	0.00	1.25
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	5.95	1.61	#DIV/0!	3.34
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.34	0.00	#NUM!	1.42
F	1	1	2-#	1					0.00221	0.002432	0.0082445	0.130402	0.0021416	0.002504	0	1.4912
F	1	1	2-#	2					0.003241	0.002882	0.0096969	0.102247	0.0022447	0.002887	0	2.2948
F	1	1	2-#	3					0.002149	0.002042	0.0072694	0.129116	0.0021494	0.002406	0	1.2906
F	1	0	2-#	1					0.003082	0.002702	0.0097841	0.102409	0.0018861	0.002428	0	0.481
F	1	0	2-#	2					0.003306	0.00243	0.0090005	0.100438	0.0022151	0.002438	0	0.5677
F	1	0	2-#	3					0.004062	0.003185	0.010827	0.091106	0.001885	0.002431	0	0.5528
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.01	0.12	0.00	0.00	0.00	1.69
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.53
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	24.23	17.13	14.54	13.18	2.63	9.78	#DIV/0!	31.40
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.02	0.00	0.00	#NUM!	0.60
G	1	1	2-#	1												
G	1	1	2-#	2												
G	1	1	2-#	3												
G	1	0	2-#	1												
G	1	0	2-#	2												
G	1	0	2-#	3												

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-4 CH4 (Vol%)	E2-4 Ethane (vol%)	E2-4 Ethene (vol%)	E2-4 CO2 (Vol%)	E2-5 CH4 (Vol%)	E2-5 Ethane (vol%)	E2-5 Ethene (vol%)	E2-5 CO2 (Vol%)	E2-6 CH4 (Vol%)	E2-6 Ethane (vol%)	E2-6 Ethene (vol%)	E2-6 CO2 (Vol%)
H	1	1	2-#	1					0.004233	0.00241	0	1.67125	0.0046748	0	0	2.4301
H	1	1	2-#	2					0.004033	0.002384	0	1.56124	0.0060097	0	0	2.4313
H	1	1	2-#	3					0.005121	0.002409	0	1.80112	0.0061277	0	0	2.3937
H	1	0	2-#	1					0.002033	0.003883	0	0.520039	0.0021834	0	0	2.6693
H	1	0	2-#	2					0.001967	0.002309	0	1.38921	0.0020275	0.002536	0.000224	1.293
H	1	0	2-#	3					0.002025	0.00234	0	0.426848	0.002029	0	0	1.3249
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	1.68	0.01	0.00	0.00	2.42
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.02
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	12.97	0.60	#DIV/0!	7.16	14.40	#DIV/0!	#DIV/0!	0.88
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	#NUM!	0.10	0.00	#NUM!	#NUM!	0.02
I	1	1	2-#	1	15.42333	0.0035794	0	14.2879	29.7554	0	0.000669	17.06303	35.473			19.084
I	1	1	2-#	2	24.36495	0.0028329	0.0004623	14.3317	29.28643	0	0	17.67514	31.55839			17.317
I	1	1	2-#	3	18.79418	0.0024903	0	11.0619	30.17481	0	0	18.01404	29.93935			17.555
I	1	0	2-#	1	0.001825	0.0023079	0	0.59302	0	0	0	0.557151	0	0.005149	0	0.533
I	1	0	2-#	2	0	0.0023394	0	0.77476	0	0	0	0.540603	0	0	0	0.7478
I	1	0	2-#	3	0	0.0023398	0	0.73146	0	0	0	1.31217	0	0	0	0.5687
					19.53	0.00	0.00	13.23	29.74	0.00	0.00	17.58	32.32	#DIV/0!	#DIV/0!	17.99
					4.52	0.00	0.00	1.88	0.44	0.00	0.00	0.48	2.85	#DIV/0!	#DIV/0!	0.96
					23.12	18.77	173.21	14.18	1.49	#DIV/0!	173.21	2.74	8.80	#DIV/0!	#DIV/0!	5.33
					5.11	0.00	0.00	2.12	0.50	#NUM!	0.00	0.55	3.22	#DIV/0!	#DIV/0!	1.08
Z	1	1	2-#	1												
Z	1	1	2-#	2												
Z	1	1	2-#	3												
Z	1	0	2-#	1												
Z	1	0	2-#	2												
Z	1	0	2-#	3												

Appendix P

TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-7 CH4 (Vol%)	E2-7 Ethane (vol%)	E2-7 Ethene (vol%)	E2-7 CO2 (Vol%)	E2-8 CH4 (Vol%)	E2-8 Ethane (vol%)	E2-8 Ethene (vol%)	E2-8 CO2 (Vol%)	E2-9 CH4 (Vol%)	E2-9 Ethane (vol%)	E2-9 Ethene (vol%)	E2-9 CO2 (Vol%)
A	1	1	2-#	1												
A	1	1	2-#	2												
A	1	1	2-#	3												
A	1	0	2-#	1												
A	1	0	2-#	2												
A	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
B	1	1	2-#	1												
B	1	1	2-#	2												
B	1	1	2-#	3												
B	1	0	2-#	1												
B	1	0	2-#	2												
B	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
C	1	1	2-#	1									49.41074	0	0.049095	38.3439
C	1	1	2-#	2									44.73256	0	0.053877	32.8062
C	1	1	2-#	3									50.53183	0	0.060342	34.9317
C	1	0	2-#	1									0.0003197	0.000877	0	26.8971
C	1	0	2-#	2									0.0006229	0	0	28.2014
C	1	0	2-#	3									0.0003804	0	0	23.9619
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	48.23	0.00	0.05	35.36
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	3.08	0.00	0.01	2.79
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	6.38	#DIV/0!	10.37	7.90
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	3.48	#NUM!	0.01	3.16
D	1	1	2-#	1												
D	1	1	2-#	2												
D	1	1	2-#	3												
D	1	0	2-#	1												
D	1	0	2-#	2												

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-7 CH4 (Vol%)	E2-7 Ethane (vol%)	E2-7 Ethene (vol%)	E2-7 CO2 (Vol%)	E2-8 CH4 (Vol%)	E2-8 Ethane (vol%)	E2-8 Ethene (vol%)	E2-8 CO2 (Vol%)	E2-9 CH4 (Vol%)	E2-9 Ethane (vol%)	E2-9 Ethene (vol%)	E2-9 CO2 (Vol%)
D	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
E	1	1	2-#	1	17.80202	0	0	34.8059	18.90923			29.4336	32.88117	0	0	29.4383
E	1	1	2-#	2	17.1449	0	0	34.0033	16.69073			26.9132	42.432424	0	0	35.3009
E	1	1	2-#	3	17.77179	0	0.0006039	33.5894	23.7652			34.595	23.3166	0	0	30.431
E	1	0	2-#	1	0.004156	0	0	29.3505	0	0.0047067	0.0069256	26.631	0.0006157	0	0	23.9992
E	1	0	2-#	2	0.00388	0	0	27.8235	0	0.0023121	0	29.1822	0	0	0	28.0356
E	1	0	2-#	3	0.003992	0	0	30.0017	0	0.002293	0	28.2245	0.0371627	0	0	26.7969
					17.57	0.00	0.00	34.13	19.79	#DIV/0!	#DIV/0!	30.31	32.88	0.00	0.00	31.72
					0.37	0.00	0.00	0.62	3.62	#DIV/0!	#DIV/0!	3.92	9.56	0.00	0.00	3.14
					2.11	#DIV/0!	173.21	1.81	18.28	#DIV/0!	#DIV/0!	12.92	29.07	#DIV/0!	#DIV/0!	9.89
					0.42	#NUM!	0.00	0.70	4.09	#DIV/0!	#DIV/0!	4.43	10.82	#NUM!	#NUM!	3.55
F	1	1	2-#	1	0.002541	0	0	2.18184	0.003008	0	0	2.20052	0.0014563	0	0	0.70736
F	1	1	2-#	2	0.002271	0	0	2.14539	0.00319	0	0	2.20248	0.0020004	0	0	0.78081
F	1	1	2-#	3	0.00226	0	0	2.15564	0.003722	0	0	2.20222	0.004845	0	0	0.99891
F	1	0	2-#	1	0	0	0	1.23536	0	0	0	1.24624	0	0	0	0
F	1	0	2-#	2	0	0	0	1.08848	0.001926	0	0	1.24994	0	0	0	0
F	1	0	2-#	3	0	0.00223662	0	1.24728	0.001932	0	0	1.38049	0	0	0	0
					0.00	0.00	0.00	2.16	0.00	0.00	0.00	2.20	0.00	0.00	0.00	0.83
					0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
					6.75	#DIV/0!	#DIV/0!	0.87	11.23	#DIV/0!	#DIV/0!	0.05	65.76	#DIV/0!	#DIV/0!	18.29
					0.00	#NUM!	#NUM!	0.02	0.00	#NUM!	#NUM!	0.00	0.00	#NUM!	#NUM!	0.17
G	1	1	2-#	1												
G	1	1	2-#	2												
G	1	1	2-#	3												
G	1	0	2-#	1												
G	1	0	2-#	2												
G	1	0	2-#	3												

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-7 CH4 (Vol%)	E2-7 Ethane (vol%)	E2-7 Ethene (vol%)	E2-7 CO2 (Vol%)	E2-8 CH4 (Vol%)	E2-8 Ethane (vol%)	E2-8 Ethene (vol%)	E2-8 CO2 (Vol%)	E2-9 CH4 (Vol%)	E2-9 Ethane (vol%)	E2-9 Ethene (vol%)	E2-9 CO2 (Vol%)
H	1	1	2-#	1	0			1.80668	0.011878	0	0.002155	1.04187				
H	1	1	2-#	2	0			1.74234	0.010892	0.0001173	0.0039218	0.97691				
H	1	1	2-#	3	0			1.96303	0.017082	0.062845	0.0046789	1.05846				
H	1	0	2-#	1	0			0.51026	0	0.0004527	0	0				
H	1	0	2-#	2	0			0.53414	0	0.0002219	0	0				
H	1	0	2-#	3	0			0.69357	0	0.0603073	0	0				
					0.00	#DIV/0!	#DIV/0!	1.84	0.01	0.02	0.00	1.03	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					0.00	#DIV/0!	#DIV/0!	0.11	0.00	0.04	0.00	0.04	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	6.18	25.04	172.72	36.12	4.20	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#NUM!	#DIV/0!	#DIV/0!	0.09	0.00	0.04	0.00	0.05	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
I	1	1	2-#	1	35.16134	9.3454E-05	0	18.7798	33.54253	0	0	17.0253				
I	1	1	2-#	2	35.77803	8.3322E-05	0	19.2133	33.16877	0	0	17.525				
I	1	1	2-#	3	34.36995	6.2517E-05	0	19.105								
I	1	0	2-#	1	0	0.0001579	0.0011512	0	0	0.0001377	0.0011399	0				
I	1	0	2-#	2	0	0.00019775	0	0	0	0.0001573	0	0				
I	1	0	2-#	3	0	0.00021171	0	0	0	0.000263	0	0				
					35.10	0.00	0.00	19.03	33.36	0.00	0.00	17.28	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					0.71	0.00	0.00	0.23	0.26	0.00	0.00	0.35	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					2.01	19.77	#DIV/0!	1.19	0.79	#DIV/0!	#DIV/0!	2.05	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					0.80	0.00	#NUM!	0.26	0.37	#NUM!	#NUM!	0.49	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Z	1	1	2-#	1	0.00956	0.0246508	0.0511833	0.25389					0.0080928	0.018411	0.043561	1.13271
Z	1	1	2-#	2	0.00955	0.0249656	0.0542051	0.24271					0.0079378	0.0198864	0.040973	1.11971
Z	1	1	2-#	3	0.00898	0.0239634	0.0477325	0.26599					0.0080207	0.0210617	0.046045	0.27742
Z	1	0	2-#	1	0.005162	0.00639051	0.0046899	0.26118					0.0044532	0.010542	0.019673	0.33121
Z	1	0	2-#	2	0.003651	0.00850022	0.0088966	1.10019					0.0067038	0.0157299	0.034382	0.2755
Z	1	0	2-#	3	0.004741	0.0124421	0.0182282	1.10769					0.0024249	0.0033746	0	0.30694
					0.01	0.02	0.05	0.25					0.01	0.02	0.04	0.84
					0.00	0.00	0.00	0.01					0.00	0.00	0.00	0.49
					3.54	2.09	6.35	4.58					0.97	6.71	5.83	58.12
					0.00	0.00	0.00	0.01					0.00	0.00	0.00	0.55

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-10 CH4 (Vol%)	E2-10 Ethane (vol%)	E2-10 Ethene (vol%)	E2-10 CO2 (Vol%)	E2-11 CH4 (Vol%)	E2-11 Ethane (vol%)	E2-11 Ethene (vol%)	E2-11 CO2 (Vol%)	E2-12 CH4 (Vol%)	E2-12 Ethane (vol%)	E2-12 Ethene (vol%)	E2-12 CO2 (Vol%)
A	1	1	2-#	1												
A	1	1	2-#	2												
A	1	1	2-#	3												
A	1	0	2-#	1												
A	1	0	2-#	2												
A	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
B	1	1	2-#	1					0.0047228	0	0	0.2				
B	1	1	2-#	2					0.0067902	0	0	0.181098				
B	1	1	2-#	3					0.0005852	0	0	0.396367				
B	1	0	2-#	1					0.0003652	0	0	0				
B	1	0	2-#	2					0.0006187	0	0	0				
B	1	0	2-#	3					0.0050589	0	0	0.146725				
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.26	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.12	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	78.35	#DIV/0!	#DIV/0!	46.00	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	#NUM!	#NUM!	0.13	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
C	1	1	2-#	1	11.43529	0	0	33.57655	35.70283	0	0.0009435	33.5011	28.62675	0.002933	0.0033642	22.99955
C	1	1	2-#	2	4.25696	0	0	32.03789	26.43901	0	0.0056292	31.6998	39.35558	0	0.0022757	32.62107
C	1	1	2-#	3	2.95608	0	0	32.35144	31.22479	0	0	33.6501	31.54188	0.002922	0.0021921	27.84452
C	1	0	2-#	1	0.0132141	0	0	29.52646	0.0003193	0.0008618	0	26.93614	0.001855	0.002876	0	22.11406
C	1	0	2-#	2	0.0006219	0	0	28.9415	0.0003623	0	0	23.33601	0.002	0.003	0	23.29465
C	1	0	2-#	3	0.0006602	0	0	27.2371	0.0005677	0	0	29.0681	0.002	0.000	0	27.04496
					6.22	0.00	0.00	32.66	31.12	0.00	0.00	32.95	33.17	0.00	0.00	27.82
					4.57	0.00	0.00	0.81	4.63	0.00	0.00	1.09	5.55	0.00	0.00	4.81
					73.46	#DIV/0!	#DIV/0!	2.49	14.89	#DIV/0!	137.61	3.29	16.72	86.60	25.05	17.29
					5.17	#NUM!	#NUM!	0.92	5.24	#NUM!	0.00	1.23	6.28	0.00	0.00	5.44
D	1	1	2-#	1												
D	1	1	2-#	2												
D	1	1	2-#	3												
D	1	0	2-#	1												
D	1	0	2-#	2												

Appendix P

TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-10 CH_4 (Vol%)	E2-10 Ethane (vol%)	E2-10 Ethene (vol%)	E2-10 CO_2 (Vol%)	E2-11 CH_4 (Vol%)	E2-11 Ethane (vol%)	E2-11 Ethene (vol%)	E2-11 CO_2 (Vol%)	E2-12 CH_4 (Vol%)	E2-12 Ethane (vol%)	E2-12 Ethene (vol%)	E2-12 CO_2 (Vol%)
D	1	0	2-#	3												
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
E	1	1	2-#	1	25.97227	0	0	30.92687	35.415455	0	0	3.94383				
E	1	1	2-#	2	28.59711	0	0	34.30411	37.05545	0	0	38.76864	34.920849	0	0.0004227	36.26113
E	1	1	2-#	3	29.94321	0	0	35.136	22.730126	0	0	30.97008	34.182645	0	0.0004242	35.76384
E	1	0	2-#	1	0.0017998	0	0	25.95471	0.434535	0	0	21.71537	0.023875	0	0	19.75167
E	1	0	2-#	2	0	0	0	17.12695	0.122804	0	0	26.8411	0.614589	0	0	24.13367
E	1	0	2-#	3	0.0402539	0	0	22.08293	0.0045798	0	0	27.8907	1.63747	0	0	23.28036
					28.17	0.00	0.00	33.46	31.73	0.00	0.00	24.56	34.55	0.00	0.00	36.01
					2.02	0.00	0.00	2.23	7.84	0.00	0.00	18.28	0.52	0.00	0.00	0.35
					7.17	#DIV/0!	#DIV/0!	6.66	24.71	#DIV/0!	#DIV/0!	74.41	1.51	#DIV/0!	0.24	0.98
					2.29	#NUM!	#NUM!	2.52	8.87	#NUM!	#NUM!	20.68	0.72	#NUM!	0.00	0.49
F	1	1	2-#	1					0.0062297	0	0	0.345781				
F	1	1	2-#	2					0.0005445	0	0	0				
F	1	1	2-#	3					0.0051766	0	0	0.395632				
F	1	0	2-#	1					0.0004254	0	0	0				
F	1	0	2-#	2					0.0089486	0	0	0.026353				
F	1	0	2-#	3					0.0005452	0	0	0				
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.25	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	0.00	0.00	0.22	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	75.93	#DIV/0!	#DIV/0!	87.19	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.00	#NUM!	#NUM!	0.24	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
G	1	1	2-#	1					3.22121	0	0	2.96346	5.61986	0.000	0.002	2.92637
G	1	1	2-#	2					5.95411	0	0	2.98874	5.34753	0.000	0.002	2.67144
G	1	1	2-#	3					5.33066	0	0.0009441	3.49855	5.2929	0.000	0.002	2.6046
G	1	0	2-#	1					0.0001344	0	0	0.548783	0.002	0.004	0.002	0.315055
G	1	0	2-#	2									0.000	0.000	0.002	0.351027
G	1	0	2-#	3					0.0001395	0	0	0.515888	0.000	0.000	0.002	0.369532
									4.84	0.00	0.00	3.15	5.42	0.00	0.00	2.73
									1.43	0.00	0.00	0.30	0.18	0.00	0.00	0.17
									29.62	#DIV/0!	173.21	9.58	3.23	#DIV/0!	0.19	6.21
									1.62	#NUM!	0.00	0.34	0.20	#NUM!	0.00	0.19

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-10 CH4 (Vol%)	E2-10 Ethane (vol%)	E2-10 Ethene (vol%)	E2-10 CO2 (Vol%)	E2-11 CH4 (Vol%)	E2-11 Ethane (vol%)	E2-11 Ethene (vol%)	E2-11 CO2 (Vol%)	E2-12 CH4 (Vol%)	E2-12 Ethane (vol%)	E2-12 Ethene (vol%)	E2-12 CO2 (
H	1	1	2-#	1	0.162247	0	0.081905	1.8809	1.87582	0	0.0832494	4.49868				
H	1	1	2-#	2	0.0815895	0	0.076757	1.8052	3.6156	0	0.0870531	6.99577				
H	1	1	2-#	3	0.0004864	0	0	0	4.00622	0.0012506	0.0090884	7.40433				
H	1	0	2-#	1	0.0005348	0	0	0	0.0003536	0.0009132	0	0.515012				
H	1	0	2-#	2	0.0006036	0	0	0	0.0004231	0.0009027	0	0.637495				
H	1	0	2-#	3	0.18154	0.0007654	0.078559	1.16319	0.00041	0.0009177	0	0.511481				
					0.08	0.00	0.05	1.23	3.17	0.00	0.06	6.30	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					0.08	0.00	0.05	1.06	1.13	0.00	0.04	1.57	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					99.31	#DIV/0!	86.74	86.66	35.82	173.21	73.51	24.97	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					0.09	#NUM!	0.05	1.20	1.28	0.00	0.05	1.78	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
I	1	1	2-#	1	40.090861	0	0	23.24109	48.19399	0.0009676	0.0013077	26.79479				
I	1	1	2-#	2	38.146489	0	0	21.34939	43.741226	0.000926	0.0020571	25.43894	15.28374	0.000	0.066	9.88387
I	1	1	2-#	3					39.067586	0.0009651	0.0004283	20.7157	47.9985	0.000	0.037	25.83532
I	1	0	2-#	1	0.000462	0	0	0	0.0002375	0.0008683	0	0.720962	0.002	0.004	0.000	0.645654
I	1	0	2-#	2	0.0089486	0	0	0.026353	0.0012413	0	0	0.913603	0.002	0.003	0.000	0.392125
I	1	0	2-#	3	0.0005214	0	0	0	0.0002862	0	0	1.21054	0.002	0.003	0.002	0.37561
					39.12	0.00	0.00	22.30	43.67	0.00	0.00	24.32	31.64	0.00	0.05	17.86
					1.37	0.00	0.00	1.34	4.56	0.00	0.00	3.19	23.13	0.00	0.02	11.28
					3.51	#DIV/0!	#DIV/0!	6.00	10.45	2.45	64.48	13.12	73.11	#DIV/0!	38.78	63.16
					1.91	#NUM!	#NUM!	1.85	5.16	0.00	0.00	3.61	32.06	#NUM!	0.03	15.63
Z	1	1	2-#	1									0.0113709	0.030335	0.0682553	0.009129
Z	1	1	2-#	2									0.0091245	0.025018	0.0543621	0.006489
Z	1	1	2-#	3									0.0110965	0.031723	0.0638332	0.009055
Z	1	0	2-#	1									0.010999	0.031083	0.0664526	0.021269
Z	1	0	2-#	2									0.002	0.004	0.003	0.016159
Z	1	0	2-#	3									0.0093567	0.025748	0.0555018	0.016659
													0.01	0.03	0.06	0.01
													0.00	0.00	0.01	0.00
													11.64	12.19	11.42	18.28
													0.00	0.00	0.01	0.00

Appendix P

TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-13 CH4 (Vol%)	E2-13 Ethane (vol%)	E2-13 Ethene (vol%)	E2-13 CO2 (Vol%)
A	1	1	2-#	1	0.000	0.003	0.000	0.429092
A	1	1	2-#	2	0.000	0.003	0.002	0.443959
A	1	1	2-#	3	0.000	0.003	0.022	0.506564
A	1	0	2-#	1	0.000	0.003	0.000	0.228692
A	1	0	2-#	2	0.000	0.003	0.000	0.287985
A	1	0	2-#	3	0.000	0.003	0.000	0.280692
					0.00	0.00	0.01	0.46
					0.00	0.00	0.01	0.04
					#DIV/0!	2.96	150.68	8.94
					#NUM!	0.00	0.01	0.05
B	1	1	2-#	1	0.006	0.003	0.002	1.14639
B	1	1	2-#	2	0.008	0.003	0.002	1.1479
B	1	1	2-#	3	0.007	0.003	0.002	1.11004
B	1	0	2-#	1	0.002	0.003	0.002	0.369439
B	1	0	2-#	2	0.002	0.003	0.002	0.191344
B	1	0	2-#	3	0.002	0.003	0.002	0.215697
					0.01	0.00	0.00	1.13
					0.00	0.00	0.00	0.02
					14.30	0.68	1.20	1.89
					0.00	0.00	0.00	0.02
C	1	1	2-#	1	30.7398	0.003	0.054496	31.32967
C	1	1	2-#	2	35.2041	0.003	0	33.12263
C	1	1	2-#	3	36.2887	0.003	0.05035	32.58988
C	1	0	2-#	1	0.002	0.003	0	24.4506
C	1	0	2-#	2	0.002	0.003	0	22.27606
C	1	0	2-#	3	0.002	0.003	0	23.89642
					34.08	0.00	0.03	32.35
					2.94	0.00	0.03	0.92
					8.63	0.81	86.81	2.85
					3.33	0.00	0.03	1.04
D	1	1	2-#	1	0.90856	0.000	0	1.44011
D	1	1	2-#	2	0.919	0.003	0.002174	1.57022
D	1	1	2-#	3	0.954	0.003	0.0022	1.58228
D	1	0	2-#	1	0.536	0.000	0.003	0.632048
D	1	0	2-#	2	0.024	0.003	0	0.162081

Appendix P

TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-13 CH4 (Vol%)	E2-13 Ethane (vol%)	E2-13 Ethene (vol%)	E2-13 CO2 (Vol%)
D	1	0	2-#	3	0.019	0.003	0	0.154969
					0.93	0.00	0.00	1.53
					0.02	0.00	0.00	0.08
					2.55	86.60	86.61	5.15
					0.03	0.00	0.00	0.09
E	1	1	2-#	1	27.7659	0.002904	0.040787	30.93693
E	1	1	2-#	2	39.4261	0	0.02198	36.92349
E	1	1	2-#	3	34.7754	0	0.032513	34.77537
E	1	0	2-#	1	0.13155	0.002933	0	23.85218
E	1	0	2-#	2	1.53123	0.002938	0	22.05446
E	1	0	2-#	3	0.18769	0.002869	0	24.55379
					33.99	0.00	0.03	34.21
					5.87	0.00	0.01	3.03
					17.27	173.21	29.68	8.86
					6.64	0.00	0.01	3.43
F	1	1	2-#	1				
F	1	1	2-#	2				
F	1	1	2-#	3				
F	1	0	2-#	1				
F	1	0	2-#	2				
F	1	0	2-#	3				
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
G	1	1	2-#	1	5.89442	0.003	0.000	2.70438
G	1	1	2-#	2	6.19136	0.008	0.002	2.83847
G	1	1	2-#	3	5.18204	0.003	0.002	2.4619
G	1	0	2-#	1	0.002	0.007	0.005	0.330322
G	1	0	2-#	2	0.002	0.003	0.000	0.39516
G	1	0	2-#	3	0.002	0.003	0.000	0.290164
					5.76	0.00	0.00	2.67
					0.52	0.00	0.00	0.19
					9.01	0.29	86.80	7.15
					0.59	0.00	0.00	0.22

Appendix P
TCE Measurements

Treat	Nutrient	Biotic	Event	Rep	E2-13 CH4 (Vol%)	E2-13 Ethane (vol%)	E2-13 Ethene (vol%)	E2-13 CO2 (Vol%)
H	1	1	2-#	1	9.45698	0.003	0.102	7.66966
H	1	1	2-#	2	35.7999	0.003	0.078	16.25927
H	1	1	2-#	3	34.6575	0.002902	0.085032	16.35599
H	1	0	2-#	1	0.002	0.000	0.000	0.258731
H	1	0	2-#	2	0.002	0.000	0.000	0.335106
H	1	0	2-#	3	0.002	0.003	0.000	0.565668
					26.64	0.00	0.09	13.43
					14.89	0.00	0.01	4.99
					55.90	2.95	14.14	37.14
					11.91	0.00	0.01	3.99
I	1	1	2-#	1	49.7147	0.003	0.038	23.75654
I	1	1	2-#	2	37.2299	0.006	0.042	19.81883
I	1	1	2-#	3	46.1961	0.005	0.052	23.75246
I	1	0	2-#	1	0.002	0.003	0.000	0.654404
I	1	0	2-#	2	0.140	0.003	0.000	0.869358
I	1	0	2-#	3	1.444	0.003	0.000	1.1758
					44.38	0.00	0.04	22.44
					6.44	0.00	0.01	2.27
					14.51	31.59	15.85	10.12
					7.28	0.00	0.01	2.57
Z	1	1	2-#	1				
Z	1	1	2-#	2				
Z	1	1	2-#	3				
Z	1	0	2-#	1				
Z	1	0	2-#	2				
Z	1	0	2-#	3				

Appendix Q
EC Measurements

Treat	Nutrient	Biotic	Event	Rep	Microcosm No.	E2-1 DO (mg/L)	E2-2 DO (mg/L)	E2-3 DO (mg/L)	E2-4 DO (mg/L)	E2-5 DO (mg/L)	E2-6 DO (mg/L)
A	1	1	2-#	1		0.00	0.00	0.00	0.03	0.00	0.00
A	1	1	2-#	2		0.00	0.00	0.00	0.00	0.69	0.00
A	1	1	2-#	3		0.00	0.00	0.00	0.00	0.48	0.00
A	1	0	2-#	1		0.00	0.00	0.00	0.00	0.30	0.00
A	1	0	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
A	1	0	2-#	3		0.00	0.00	0.00	2.92	0.00	0.00
B	1	1	2-#	1		0.70	0.00	0.00	1.25	0.19	0.00
B	1	1	2-#	2		0.96	0.00	0.00	0.00	0.00	0.00
B	1	1	2-#	3		1.02	0.00	0.00	0.00	0.00	0.00
B	1	0	2-#	1		1.22	0.00	0.00	0.00	0.00	0.00
B	1	0	2-#	2		1.15	0.00	0.00	0.00	0.00	0.00
B	1	0	2-#	3		1.21	0.00	0.00	0.00	0.00	0.00
C	1	1	2-#	1		0.66	0.15	0.00	0.00	0.00	0.00
C	1	1	2-#	2		0.67	0.28	0.00	0.00	0.00	0.00
C	1	1	2-#	3		0.67	0.00	0.00	0.00	0.00	0.00
C	1	0	2-#	1		2.08	0.87	0.00	0.00	0.00	0.00
C	1	0	2-#	2		2.15	0.47	0.00	0.00	0.08	0.00
C	1	0	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
D	1	1	2-#	1		1.89	0.00	0.00	0.00	2.30	0.00
D	1	1	2-#	2		1.70	0.00	0.00	0.00	1.01	0.00
D	1	1	2-#	3		1.52	0.00	0.00	0.00	2.15	0.00
D	1	0	2-#	1		1.36	0.00	0.00	0.00	3.50	0.00
D	1	0	2-#	2		1.22	0.00	0.00	0.00	0.00	0.00
D	1	0	2-#	3		1.98	0.00	0.00	0.00	0.00	0.00
E	1	1	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
E	1	1	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
E	1	1	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
E	1	0	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
E	1	0	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
E	1	0	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
F	1	1	2-#	1		0.92	0.00	0.00	0.00	0.00	0.00
F	1	1	2-#	2		0.15	0.00	0.00	0.00	0.00	0.00
F	1	1	2-#	3		0.54	0.00	0.00	0.00	0.00	0.00
F	1	0	2-#	1		1.70	0.00	0.00	0.00	0.00	0.00
F	1	0	2-#	2		0.90	0.00	0.00	0.00	0.00	0.00
F	1	0	2-#	3		1.49	0.00	0.00	0.00	0.00	0.00
G	1	1	2-#	1		0.31	0.00	0.00	0.00	0.00	0.00
G	1	1	2-#	2		0.28	0.00	0.00	0.00	0.00	0.00
G	1	1	2-#	3		0.59	0.00	0.00	0.00	0.00	0.00
G	1	0	2-#	1		1.75	0.31	0.00	0.00	0.00	0.00
G	1	0	2-#	2		2.66	0.00	0.00	0.00	0.00	0.00
G	1	0	2-#	3		2.32	0.00	0.00	0.00	0.00	0.00
H	1	1	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
H	1	1	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
H	1	1	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
H	1	0	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
H	1	0	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
H	1	0	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
I	1	1	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
I	1	1	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
I	1	1	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
I	1	0	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
I	1	0	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
I	1	0	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
Z	1	1	2-#	1		0.00	0.00	0.00	0.00	1.27	0.00
Z	1	1	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
Z	1	1	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00
Z	1	0	2-#	1		0.00	0.00	0.00	0.00	0.00	0.00
Z	1	0	2-#	2		0.00	0.00	0.00	0.00	0.00	0.00
Z	1	0	2-#	3		0.00	0.00	0.00	0.00	0.00	0.00

Appendix R - Kinetic Data of OU5 (OU12) and ZVI

					Co	
OU 12 Aerobically Corroded					436	
time in contact with iron	**TCE**	**TCE**	**cis 1,2 DCE**	**cis 1,2 DCE**	**TCE**	**TCE**
(hours)	**(μg/L)**	**(mM)**	**(μg/L)**	**(mM)**	**C/Co**	**ln(C/Co)**
0.00	463.5	3.53E-03	0	0.00E+00	1.063	0.06
0.00	468.6	3.57E-03	0	0.00E+00	1.075	0.07
0.00	375.9	2.86E-03	0	0.00E+00	0.862	-0.15
4.20	72.1	5.49E-04	0	0.00E+00	0.165	-1.80
4.20	73.0	5.55E-04	0	0.00E+00	0.167	-1.79
4.15	91.4	6.96E-04	0	0.00E+00	0.210	-1.56
7.62	44.3	3.37E-04	0	0.00E+00	0.102	-2.29
7.60	50.2	3.82E-04	0	0.00E+00	0.115	-2.16
7.55	47.9	3.65E-04	0	0.00E+00	0.110	-2.21
11.87	29.8	2.27E-04	0	0.00E+00	0.068	-2.68
11.87	27.2	2.07E-04	0	0.00E+00	0.062	-2.77
10.80	31.0	2.36E-04	0	0.00E+00	0.071	-2.64
24.62	0.0	0.00E+00	0	0.00E+00	0.000	
24.60	7.9	6.03E-05	0	0.00E+00	0.018	-4.01
24.55	8.7	6.63E-05	0	0.00E+00	0.020	-3.91
27.98	0.0	0.00E+00	0	0.00E+00	0.000	
28.32	8.6	6.55E-05	0	0.00E+00	0.020	-3.92
28.65	0	0.00E+00	0	0.00E+00	0.000	
50.45	0	0.00E+00	0	0.00E+00	0.000	
50.78	0	0.00E+00	0	0.00E+00	0.000	
51.12	0	0.00E+00	0	0.00E+00	0.000	
74.18	0	0.00E+00	0	0.00E+00	0.000	
74.52	0	0.00E+00	0	0.00E+00	0.000	
74.85	0	0.00E+00	0	0.00E+00	0.000	
97.18	0	0.00E+00	0	0.00E+00	0.000	
97.52	0	0.00E+00	0	0.00E+00	0.000	
97.83	0	0.00E+00	0	0.00E+00	0.000	
					Co	
					1015	
OU 12						
Time in vial	**TCE**	**TCE**	**cis 1,2 DCE**	**cis 1,2 DCE**	**TCE**	
(hours)	**(μg/L)**	**(mM)**	**(μg/L)**	**(mM)**	**C/Co**	
0.00	1007	7.66E-03	0	0.00E+00	0.992	-0.009
0.00	1021	7.77E-03	0	0.00E+00	1.006	0.006
0.00	1018	7.75E-03	0	0.00E+00	1.003	0.003
4.15	982	7.47E-03	0	0.00E+00	0.967	-0.033
4.15	983	7.48E-03	0	0.00E+00	0.968	-0.032
4.15	1003	7.64E-03	0	0.00E+00	0.988	-0.012
7.57	1013	7.71E-03	0	0.00E+00	0.998	-0.002
7.55	1026	7.81E-03	0	0.00E+00	1.010	0.010
7.55	987.9	7.52E-03	0	0.00E+00	0.973	-0.027
11.82	995.5	7.58E-03	0	0.00E+00	0.980	-0.020
11.82	976.4	7.43E-03	0	0.00E+00	0.962	-0.039
11.80	950.4	7.23E-03	0	0.00E+00	0.936	-0.066
24.57	980.2	7.46E-03	0	0.00E+00	0.965	-0.035
24.55	996.4	7.58E-03	0	0.00E+00	0.981	-0.019
24.55	971.3	7.39E-03	0	0.00E+00	0.957	-0.044
27.98	969.3	7.38E-03	0	0.00E+00	0.955	-0.046
28.32	968.1	7.37E-03	0	0.00E+00	0.953	-0.048
28.65	924.6	7.04E-03	0	0.00E+00	0.911	-0.094
50.40	966.1	7.35E-03	0	0.00E+00	0.951	-0.050
50.73	931.7	7.09E-03	0	0.00E+00	0.918	-0.086
51.07	941.4	7.16E-03	0	0.00E+00	0.927	-0.076
74.13	853.4	6.50E-03	0	0.00E+00	0.840	-0.174
74.47	844.0	6.42E-03	0	0.00E+00	0.831	-0.185
74.80	827.5	6.30E-03	0	0.00E+00	0.815	-0.205
97.13	894.1	6.80E-03	0	0.00E+00	0.881	-0.127
97.47	817.8	6.22E-03	0	0.00E+00	0.805	-0.216
97.78	849.2	6.46E-03	0	0.00E+00	0.836	-0.179

					Co	
OU 12 Anaerobically Corroded					121	
time in contact with iron	**TCE**	**TCE**	**cis 1,2 DCE**	**cis 1,2 DCE**	**TCE**	**TCE**
hours	**(µg/L)**	**(mM)**	**(µg/L)**	**(mM)**	**C/Co**	**ln(C/Co)**
0.00	125.3	9.53E-04	0	0.00E+00	1.039	0.038
0.00	128.8	9.80E-04	0	0.00E+00	1.069	0.066
0.00	107.6	8.19E-04	0	0.00E+00	0.892	-0.114
3.92	38.4	2.92E-04	19.5	2.01E-04	0.319	-1.143
3.93	35.5	2.70E-04	19.9	2.05E-04	0.295	-1.221
3.92	34.3	2.61E-04	0.0	0.00E+00	0.285	-1.256
7.85	32.3	2.46E-04	21.9	2.26E-04	0.268	-1.317
7.85	32.1	2.45E-04	22.9	2.36E-04	0.267	-1.322
7.85	26.9	2.05E-04	20.4	2.10E-04	0.223	-1.498
11.77	24.4	1.86E-04	27.0	2.79E-04	0.203	-1.596
11.78	23.8	1.81E-04	25.1	2.59E-04	0.197	-1.624
11.78	24.9	1.89E-04	25.3	2.61E-04	0.206	-1.578
23.75	20.7	1.57E-04	30.7	3.16E-04	0.171	-1.763
23.77	19.1	1.46E-04	27.4	2.83E-04	0.159	-1.841
23.75	20.4	1.56E-04	25.6	2.64E-04	0.170	-1.775
48.15	14.7	1.12E-04	28.1	2.90E-04	0.122	-2.101
48.17	13.9	1.06E-04	35.9	3.70E-04	0.115	-2.159
48.15	14.1	1.07E-04	33.3	3.43E-04	0.117	-2.148
72.27	9.6	7.28E-05	28.5	2.94E-04	0.079	-2.533
72.28	8.7	6.60E-05	26.6	2.74E-04	0.072	-2.632
72.28	9.6	7.31E-05	30.1	3.10E-04	0.080	-2.530
97.60	6.7	5.09E-05	29.4	3.03E-04	0.055	-2.891
97.62	8.2	6.26E-05	34.6	3.57E-04	0.068	-2.685
97.62	7.2	5.46E-05	28.6	2.95E-04	0.060	-2.821
180.18	0	0.00E+00	26.2	2.70E-04	0	
180.18	0	0.00E+00	22.5	2.32E-04	0	
180.18	0	0.00E+00	0.0	0.00E+00	0	
335.80	0	0.00E+00	0.0	0.00E+00	0	
335.80	0	0.00E+00	20.6	2.12E-04	0	
335.80	0	0.00E+00	21.7	2.24E-04	0	
					Co	
OU 12					214	
time in vial	**TCE**	**TCE**	**cis 1,2 DCE**	**cis 1,2 DCE**	**TCE**	
hours	**(µg/L)**	**(mM)**	**(µg/L)**	**(mM)**	**C/Co**	
0.00	219.8	1.67E-03	0	0.00E+00	1.029	0.028
0.00	217.0	1.65E-03	0	0.00E+00	1.015	0.015
0.00	204.4	1.56E-03	0	0.00E+00	0.956	-0.045
3.93	221.1	1.68E-03	0	0.00E+00	1.035	0.034
3.93	209.7	1.60E-03	0	0.00E+00	0.981	-0.019
3.92	213.5	1.62E-03	0	0.00E+00	0.999	-0.001
7.87	223.5	1.70E-03	0	0.00E+00	1.046	0.045
7.85	214.2	1.63E-03	0	0.00E+00	1.002	0.002
7.85	211.8	1.61E-03	0	0.00E+00	0.991	-0.009
11.78	230.6	1.75E-03	0	0.00E+00	1.079	0.076
11.78	215.6	1.64E-03	0	0.00E+00	1.009	0.009
11.78	201.5	1.53E-03	0	0.00E+00	0.943	-0.059
23.77	217.6	1.66E-03	0	0.00E+00	1.018	0.018
23.77	213.4	1.62E-03	0	0.00E+00	0.998	-0.002
23.75	209.3	1.59E-03	0	0.00E+00	0.979	-0.021
48.17	225.1	1.71E-03	0	0.00E+00	1.053	0.052
48.17	215.4	1.64E-03	0	0.00E+00	1.008	0.008
48.15	202.4	1.54E-03	0	0.00E+00	0.947	-0.054
72.28	219.7	1.67E-03	0	0.00E+00	1.028	0.028
72.28	208.3	1.59E-03	0	0.00E+00	0.974	-0.026
72.27	209.8	1.60E-03	0	0.00E+00	0.982	-0.019
97.62	215.4	1.64E-03	0	0.00E+00	1.008	0.008
97.62	210.2	1.60E-03	0	0.00E+00	0.983	-0.017
97.62	200.9	1.53E-03	0	0.00E+00	0.940	-0.062
179.22	218.3	1.66E-03	0	0.00E+00	1.022	0.021
179.20	202.0	1.54E-03	0	0.00E+00	0.945	-0.056
179.20	186.7	1.42E-03	0	0.00E+00	0.874	-0.135
335.82	189.2	1.44E-03	0	0.00E+00	0.885	-0.122
335.80	183.4	1.40E-03	0	0.00E+00	0.858	-0.153
335.80	176.8	1.35E-03	0	0.00E+00	0.827	-0.189

OU 12 Uncorroded time in contact with iron hours	TCE (µg/L)	TCE (mM)	cis 1,2 DCE (µg/L)	cis 1,2 DCE (mM)	Co 434 TCE C/C0	TCE ln (C/Co)
0.00	439.6	3.35E-03	0	0.00E+00	1.012	0.012
0.00	407.5	3.10E-03	0	0.00E+00	0.938	-0.064
0.00	456.2	3.47E-03	0	0.00E+00	1.050	0.049
4.0	381.1	2.90E-03	0	0.00E+00	0.877	-0.131
4.0	358.2	2.73E-03	0	0.00E+00	0.825	-0.193
4.0	355.2	2.70E-03	0	0.00E+00	0.818	-0.201
7.7	353.8	2.69E-03	0	0.00E+00	0.814	-0.205
7.7	324.6	2.47E-03	26.6	2.74E-04	0.747	-0.291
7.7	354.9	2.70E-03	28.1	2.90E-04	0.817	-0.202
13.6	303.1	2.31E-03	45.8	4.72E-04	0.698	-0.360
13.6	330.2	2.51E-03	38.6	3.98E-04	0.760	-0.274
13.6	327.3	2.49E-03	43.0	4.44E-04	0.753	-0.283
24.1	256.6	1.95E-03	64.8	6.68E-04	0.591	-0.526
24.1	266.1	2.03E-03	60.1	6.20E-04	0.613	-0.490
24.1	255.0	1.94E-03	67.4	6.95E-04	0.587	-0.533
50.7	174.9	1.33E-03	102	1.05E-03	0.403	-0.910
50.7	173.9	1.32E-03	102	1.05E-03	0.400	-0.915
51.8	169.1	1.29E-03	102	1.06E-03	0.389	-0.943
75.0	126.2	9.61E-04	126	1.30E-03	0.291	-1.236
75.0	118.1	8.99E-04	120	1.24E-03	0.272	-1.302
75.0	122.3	9.31E-04	119	1.22E-03	0.282	-1.267
99.2	101.4	7.72E-04	138	1.42E-03	0.234	-1.455
99.2	94.1	7.16E-04	132	1.36E-03	0.217	-1.529
99.2	88.7	6.75E-04	129	1.34E-03	0.204	-1.589
171	38.2	2.91E-04	133	1.37E-03	0.088	-2.432
171	39.4	3.00E-04	142	1.47E-03	0.091	-2.401
171	39.5	3.00E-04	138	1.43E-03	0.091	-2.399
294	11.7	8.87E-05	125	1.29E-03	0.027	-3.619
295	11.5	8.71E-05	116	1.20E-03	0.026	-3.636
295	12.6	9.57E-05	129	1.33E-03	0.029	-3.542

OU 12 Time in vial hours	TCE (µg/L)	TCE (mM)	cis 1,2 DCE (µg/L)	cis 1,2 DCE (mM)	Co 469 TCE C/C0	TCE ln (C/Co)
0.00	490	3.73E-03	0	0.00E+00	1.044	0.043
0.00	444	3.38E-03	0	0.00E+00	0.947	-0.055
0.00	474	3.60E-03	0	0.00E+00	1.009	0.009
4.02	483	3.68E-03	0	0.00E+00	1.030	0.029
4.02	468	3.56E-03	0	0.00E+00	0.998	-0.002
4.02	476	3.62E-03	0	0.00E+00	1.015	0.014
7.65	402	3.06E-03	0	0.00E+00	0.857	-0.154
7.65	481	3.66E-03	0	0.00E+00	1.024	0.024
7.67	444	3.38E-03	0	0.00E+00	0.946	-0.055
13.62	496	3.78E-03	0	0.00E+00	1.058	0.056
13.62	369	2.81E-03	0	0.00E+00	0.786	-0.241
13.63	458	3.48E-03	0	0.00E+00	0.975	-0.025
24.07	356	2.71E-03	0	0.00E+00	0.759	-0.276
24.07	485	3.69E-03	0	0.00E+00	1.034	0.033
24.07	443	3.37E-03	0	0.00E+00	0.945	-0.057
50.67	459	3.49E-03	0	0.00E+00	0.979	-0.022
50.83	476	3.62E-03	0	0.00E+00	1.015	0.015
49.70	466	3.55E-03	0	0.00E+00	0.994	-0.006
74.95	421	3.20E-03	0	0.00E+00	0.897	-0.108
74.95	470	3.58E-03	0	0.00E+00	1.002	0.002
74.95	437	3.32E-03	0	0.00E+00	0.930	-0.072
99.23	485	3.69E-03	0	0.00E+00	1.033	0.032
99.23	451	3.43E-03	0	0.00E+00	0.961	-0.040
99.25	439	3.34E-03	0	0.00E+00	0.937	-0.066
170.82	431	3.28E-03	0	0.00E+00	0.918	-0.086
171.15	463	3.52E-03	0	0.00E+00	0.987	-0.013
171.48	422	3.21E-03	0	0.00E+00	0.899	-0.106
294.47	412	3.14E-03	0	0.00E+00	0.878	-0.130
294.80	459	3.50E-03	0	0.00E+00	0.979	-0.022
295.12	407	3.09E-03	0	0.00E+00	0.867	-0.143

Appendix S. C/Co versus Time for each ZVI Treatment

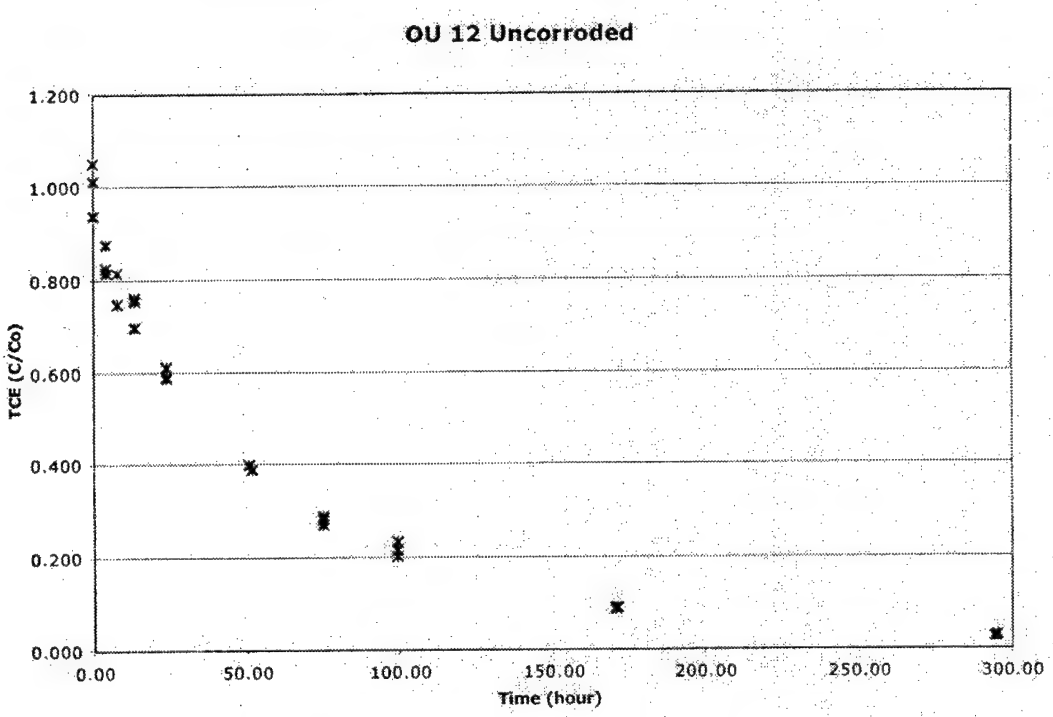
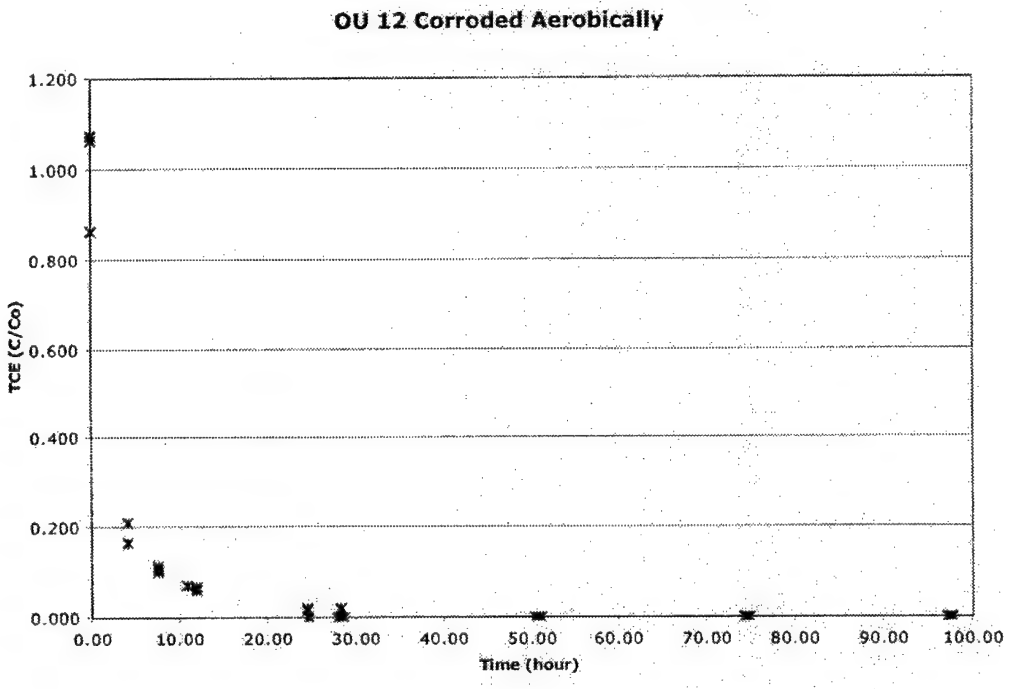


Figure 1S. Loss of TCE (C/Co) over time for untreated ZVI.

Figure 2S. Loss of TCE (C/Co) over time for aerobically pre-corroded ZVI.



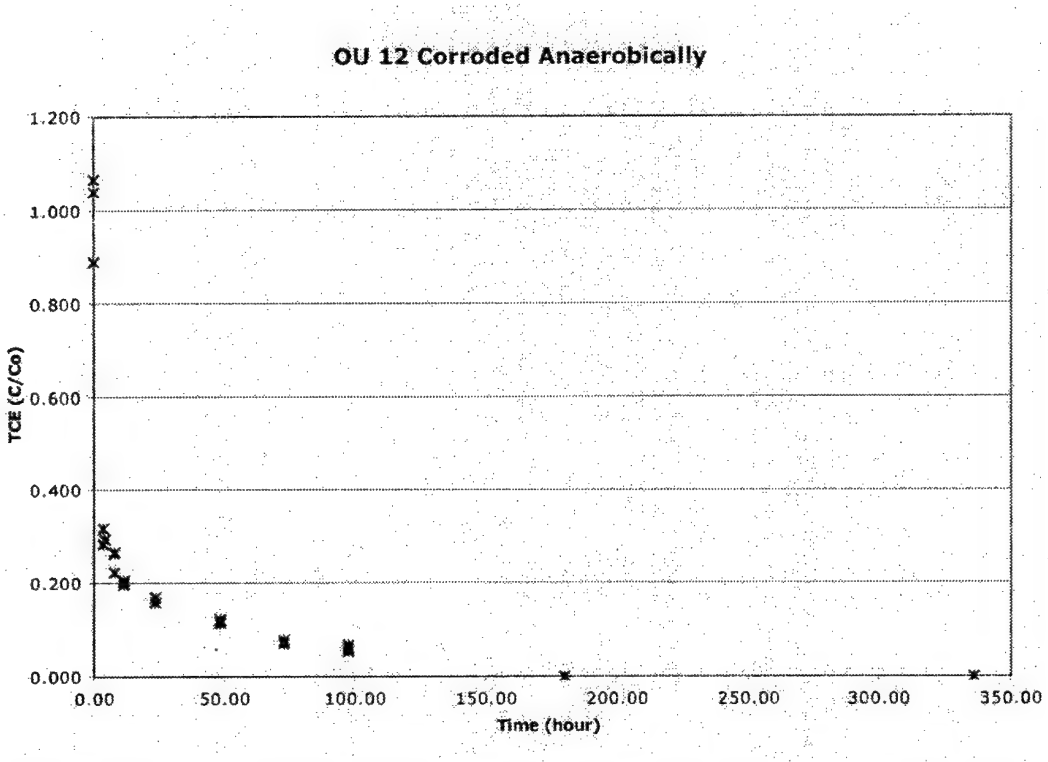


Figure 3S. Loss of TCE (C/Co) over time for anaerobically pre-corroded ZVI.

Appendix T - First-Order Kinetic Plots (ln (C/Co) versus Time) for Each ZVI Treatment

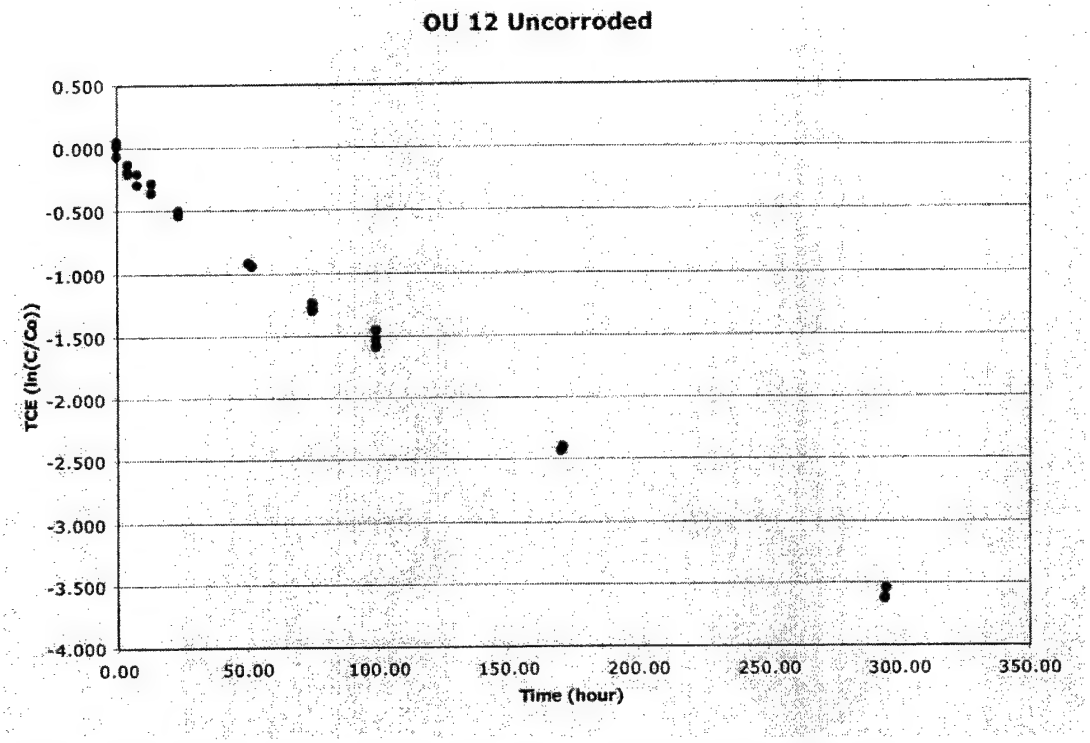


Fig 1T. First order kinetic plot for TCE loss in contact with untreated ZVI.

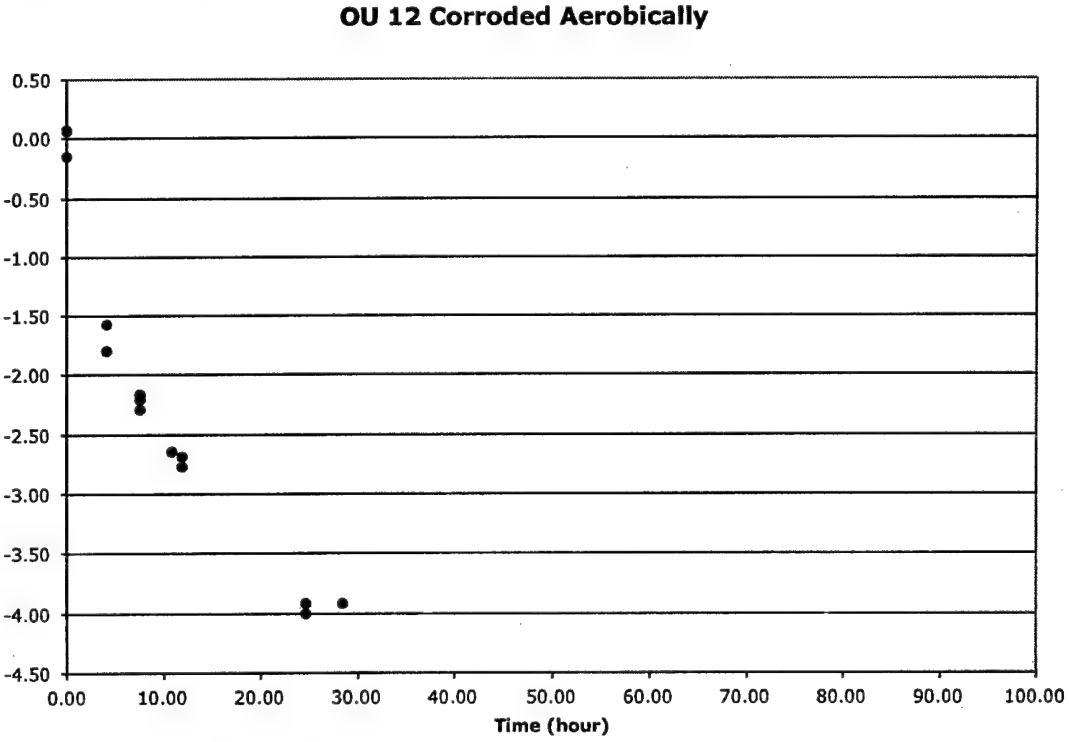


Fig 2T, First order kinetic plot for TCE loss in contact with aerobically pre-corroded ZVI.

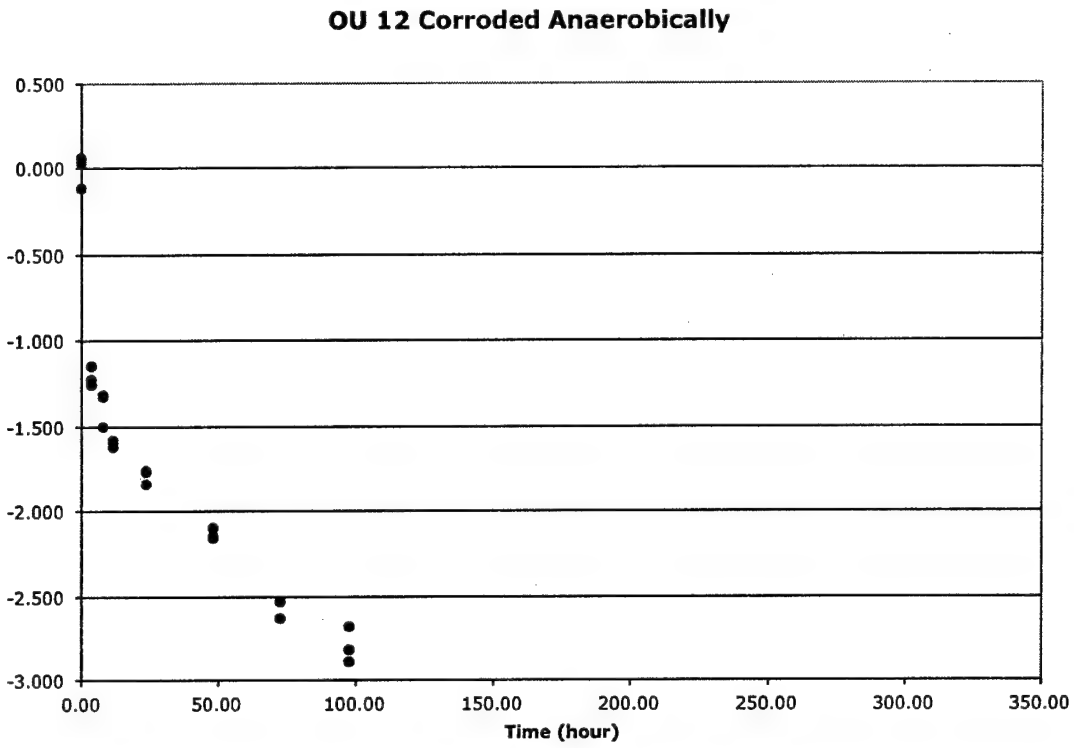


Fig 3T. First order kinetic plot for TCE loss in contact with anaerobically pre-corroded ZVI.

Appendix U. Output from S-PLUS using Nonlinear r Regression Model to Describe Two Simultaneous First-Order Reactions for Each Treatment

Uncorroded OU 12
Convergence Tolerance 0.001
Confidence Level 0.95

*** Nonlinear Regression Model ***

Formula: conc ~ c1 * exp(- k1 * time) + c2 * exp(- k2 * time)

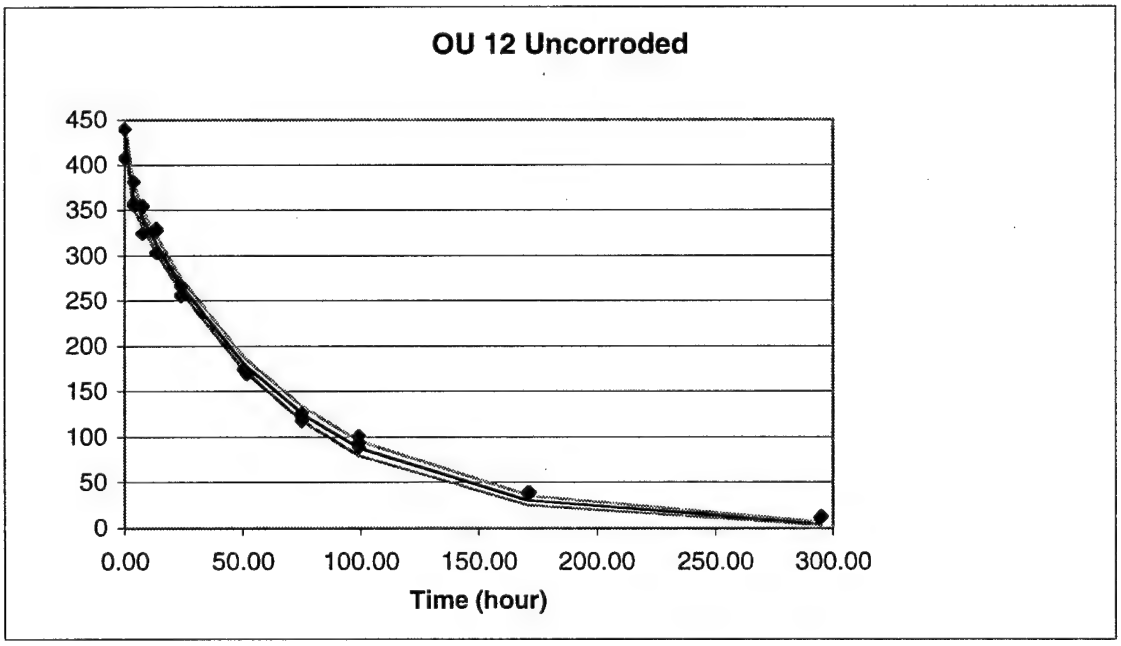
Parameters:

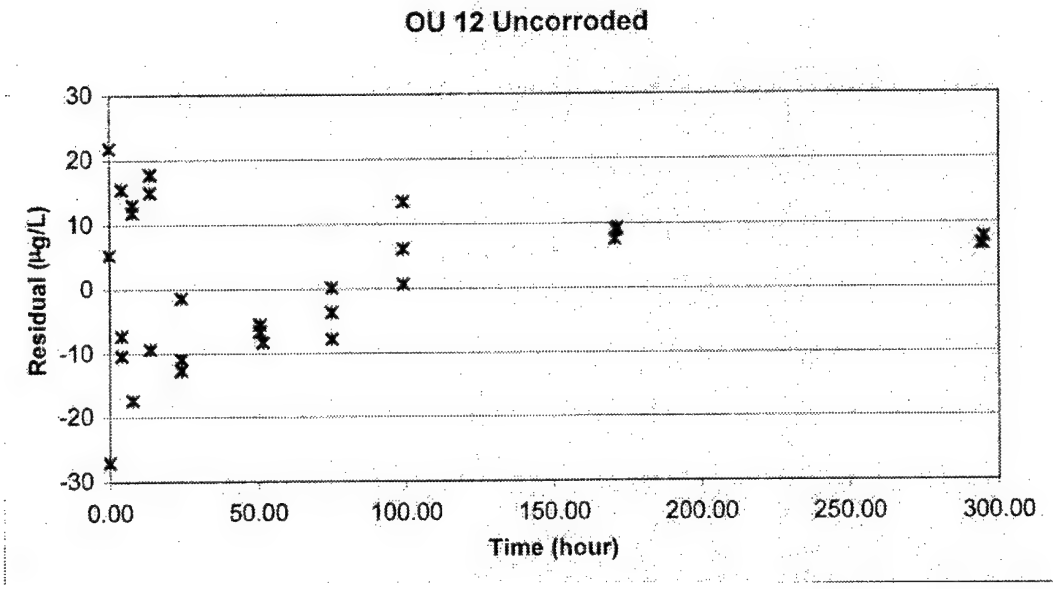
	Value	Std. Error	t value
c1	382.0590000	7.653850000	49.91730
k1	0.0147948	0.000575973	25.68660
c2	52.3283000	10.420700000	5.02157
k2	0.5561320	0.434765000	1.27916

Residual standard error: 12.352 on 26 degrees of freedom

Correlation of Parameter Estimates:

	c1	k1	c2
k1	0.743		
c2	-0.729	-0.543	
k2	0.698	0.465	-0.457




OU 12 Uncorroded
Residual (µg/L)
30
20
10
0
-10
-20
-30
0.00
50.00
100.00
150.00
200.00
250.00
300.00
Time (hour)

Aerobically Pre-Corroded OU 12
Convergence Tolerance 0.001
Confidence Level 0.95

*** Nonlinear Regression Model ***

Formula: conc ~ c1 * exp(- k1 * time) + c2 * exp(- k2 * time)

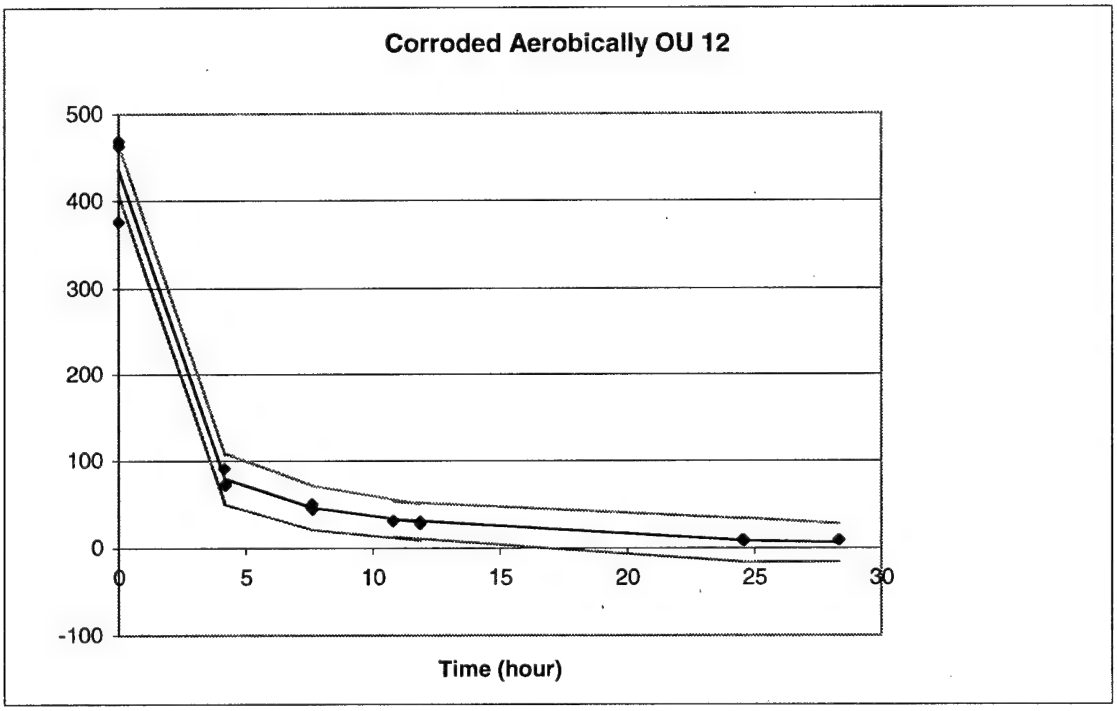
Parameters:

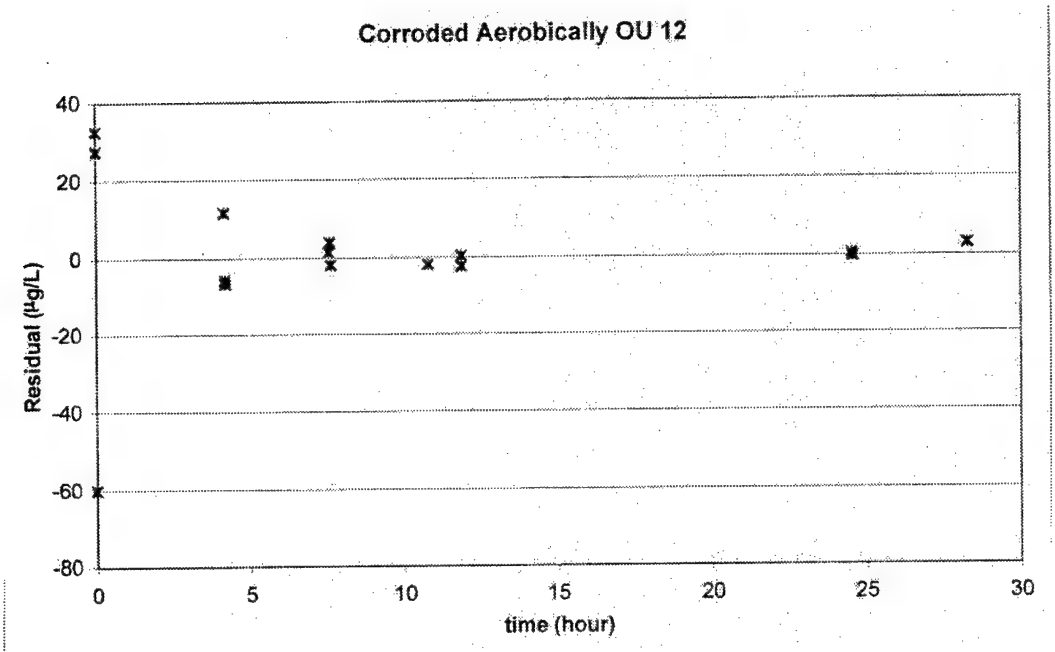
	Value	Std. Error	t value
c1	339.8090000	97.1553000	3.497590
k1	0.7352890	0.5981930	1.229180
c2	96.2136000	96.2901000	0.999206
k2	0.0997241	0.0947132	1.052910

Residual standard error: 22.7281 on 11 degrees of freedom

Correlation of Parameter Estimates:

	c1	k1	c2
k1	-0.909		
c2	-0.991	0.920	
k2	-0.952	0.824	0.960




Corroded Aerobically OU 12
Residual (µg/L)
40
20
0
-20
-40
-60
-80
0
5
10
15
20
25
30
time (hour)

Anaerobically Pre-Corroded OU 12
Convergence Tolerance 0.001
Confidence Level 0.95

*** Nonlinear Regression Model ***

Formula: conc ~ c1 * exp(- k1 * time) + c2 * exp(- k2 * time)

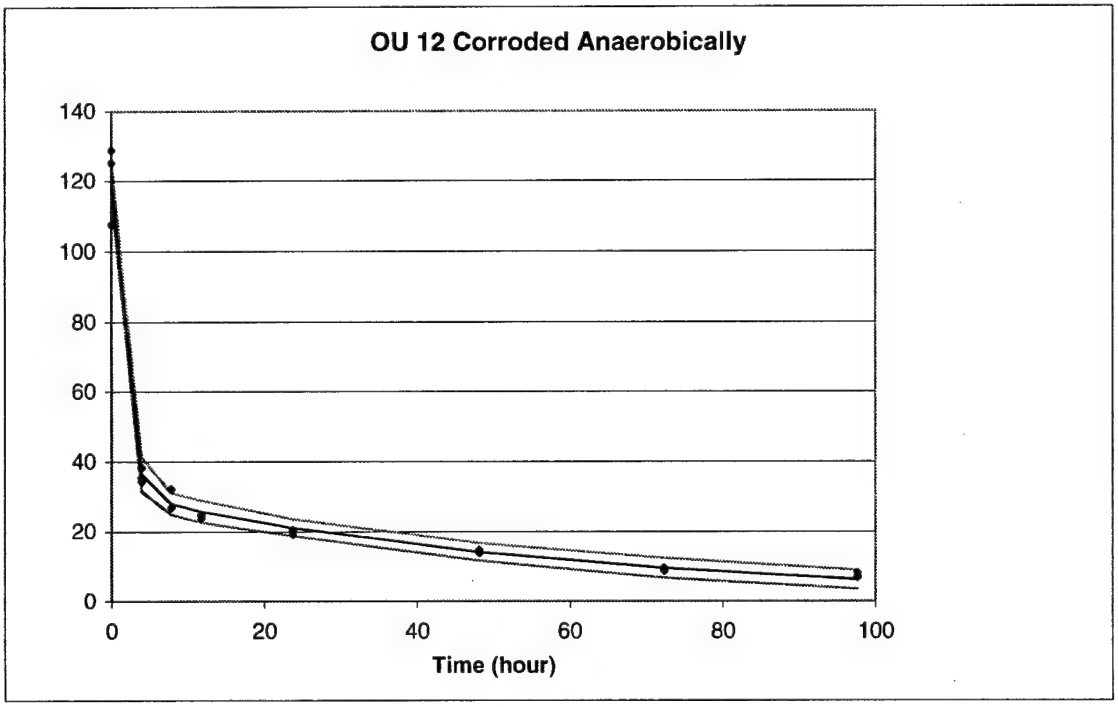
Parameters:

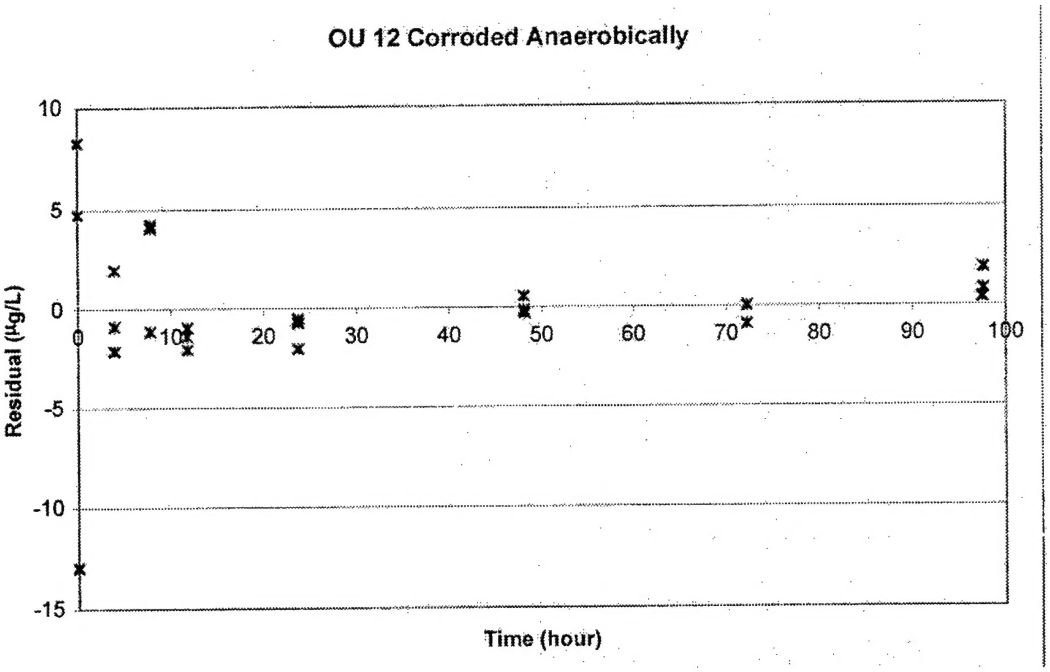
	Value	Std. Error	t value
c1	89.2454000	3.38783000	26.34290
k1	0.6444500	0.11295000	5.70561
c2	31.2841000	2.48084000	12.61030
k2	0.0164317	0.00267237	6.14873

Residual standard error: 4.01518 on 20 degrees of freedom

Correlation of Parameter Estimates:

	c1	k1	c2
k1	-0.457		
c2	-0.729	0.681	
k2	-0.545	0.451	0.747




OU 12 Corroded Anaerobically
Residual (µg/L)
Time (hour)
10
5
0
-5
-10
-15
0
10
20
30
40
50
60
70
80
90
100

Appendix V. Plots of Loss of TCE (mM) and Production of cis-DCE (mM) for Each ZVI Treatment

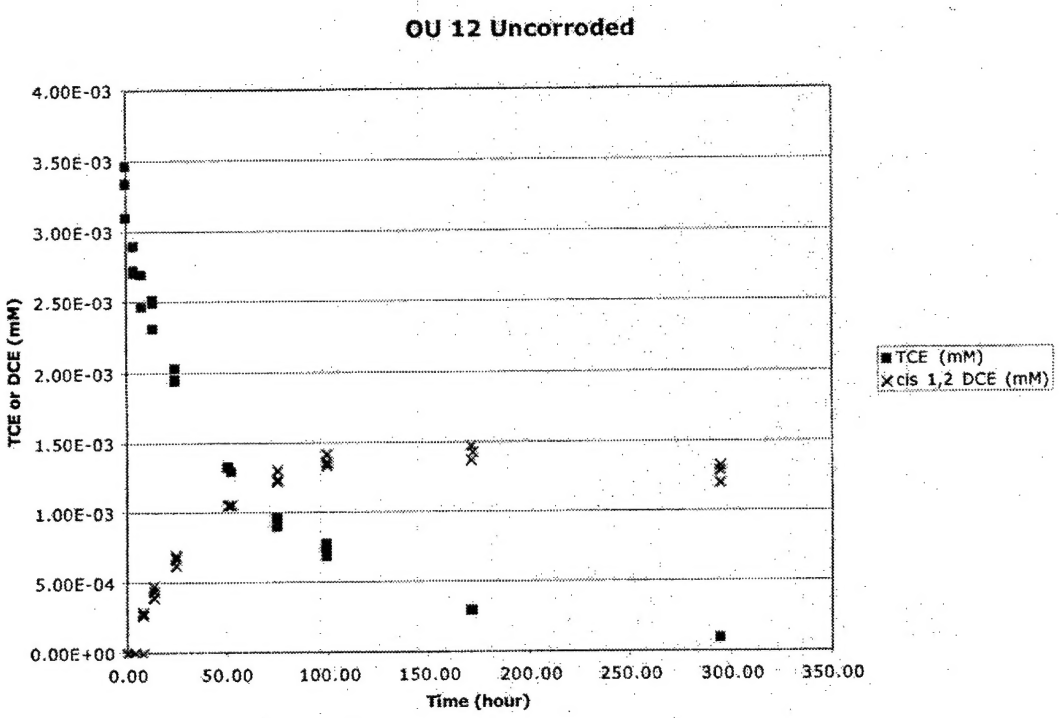


Fig 1V. Loss of TCE (mM) and production of cis-DCE (mM) over time in contact with untreated ZVI.

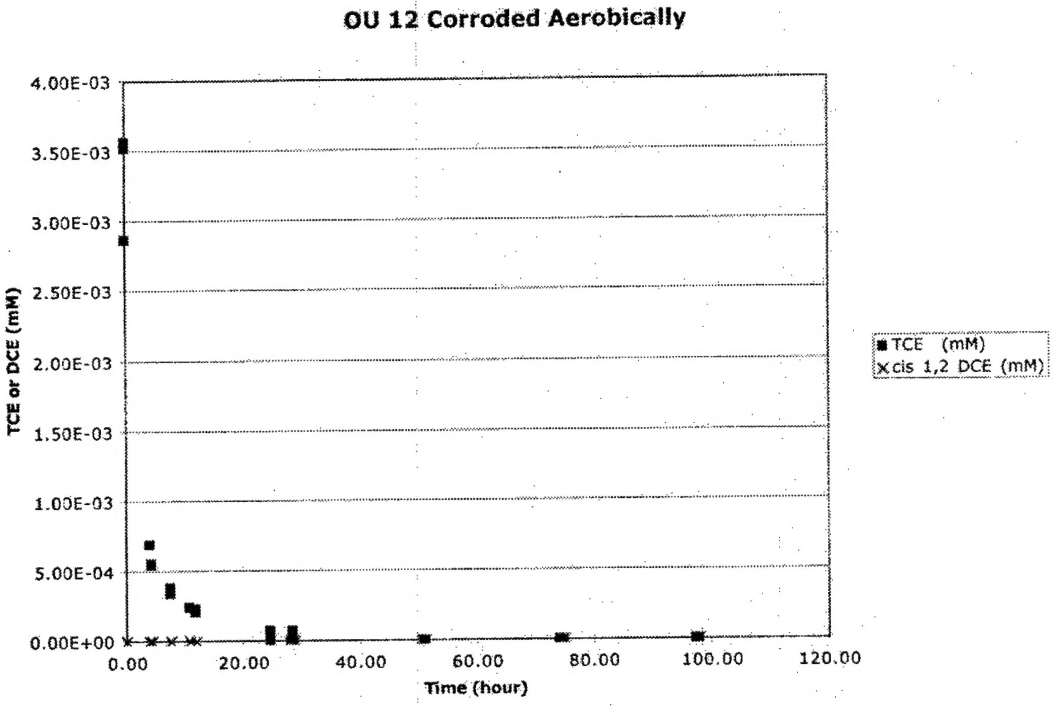


Fig 2V. Loss of TCE (mM) and production of cis-DCE (mM) over time in contact with aerobically pre-corroded ZVI.

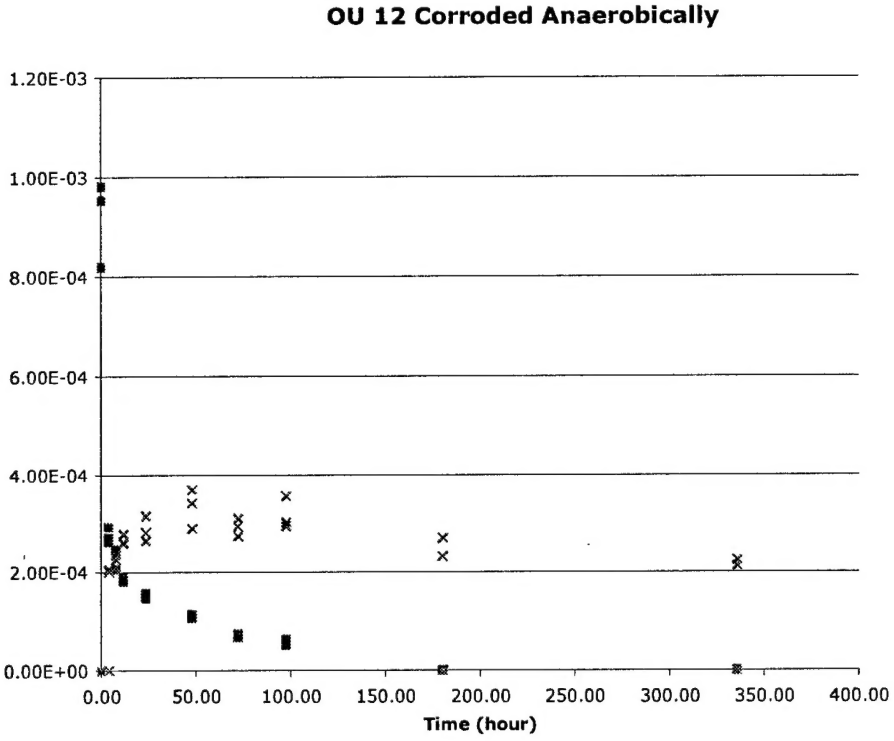


Fig 3V. Loss of TCE (mM) and production of cis-DCE (mM) over time in contact with anaerobically pre-corroded ZVI.